A 12

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 April 2002 (11.04.2002)

PCT

(10) International Publication Number
WO 02/29001 A2

(51) International Patent Classification[7]: C12N

(21) International Application Number: PCT/US01/30936

(22) International Filing Date: 3 October 2001 (03.10.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/237,436 3 October 2000 (03.10.2000) US
60/264,046 25 January 2001 (25.01.2001) US
60/297,507 13 June 2001 (13.06.2001) US

(71) Applicant (*for all designated States except US*): UNIVERSITY OF VIRGINIAN PATENT FOUNDATION [US/US]; 1224 West Main Street, Suites 1-110, Charlottesville, VA 22903 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): LYNCH, Kevin, R. [US/US]; 1632 Meadowbrook Heights Road, Charlottesville, VA 22901 (US). MACDONALD, Timothy, L. [US/US]; 2625 Jefferson Park Circle, Charlottesville, VA 22903 (US). HEISE, Christopher, E. [US/US]; 1800 Jefferson Park Avenue, Charlottesville, VA 22903 (US). SANTOS, Webster, L. [US/US]; 112-A Dunova Court, Charlottesville, VA 22903 (US). OKUSA, Mark, D. [US/US]; 2250 Cornwall Road, Charlottesville, VA 22901 (US).

(74) Agent: BREEN, John, P.; University of Virginia Patent Foundation, 1224 West Main Street, Suite 1-110, Charlottesville, VA 22903 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/29001 A2

(54) Title: NOVEL LYSOPHOSPHATIDIC ACID RECEPTOR AGONISTS AND ANTAGONISTS

(57) Abstract: The present invention is directed to compositions comprising lysophosphatidic acid analogs and methods of using such analogs as agonist or antagonists of LPA receptor activity. In addition the invention is directed to LPA receptor agonists that vary in the degree of selectivity at individual LPA receptors (i.e. LPA1, LPA2 and LPA3). More particularly the present invention is directed to LPA analogs wherein the glycerol is replaced with ethanolamine and a variety of substitutions have been linked at the second carbon atom.

Novel Lysophosphatidic Acid Receptor Agonists and Antagonists

This application claims priority under 35 U.S.C. §119(e) to provisional patent application nos. 60/237,436 filed October 3, 2000; 60/264,046 filed January 25, 2001; and 60/297,507 filed June 13, 2001, the disclosures of which are incorporated herein.

US Government Rights

This invention was made with United States Government support under Grant Nos. NIH R01 GM52722, R01 CA88994, T32 GM07055 and NIH F31 DA05927 awarded by National Institutes of Health. The United States Government has certain rights in the invention.

Field of the Invention

The present invention is directed to compositions comprising lysophosphatidic acid analogs and methods of using such analogs as agonist or antagonists of LPA receptor activity. In addition the invention is directed to LPA receptor agonists that vary in the degree of selectivity at individual LPA receptors (i.e. LPA1, LPA2 and LPA3). Selective agonists and antagonists at LPA receptors will be useful therapeutically in a wide variety of human disorders.

Background of the Invention

Lysophosphatidic acid (LPA, 1-acyl, 2-hydroxyl-*sn*-glycerol-3-phosphate) is an intermediary metabolite in all cells but is released from some cells to act as a mediator that elicits a wide variety of responses from cells/tissues. These responses include calcium mobilization, cytoskeletal rearrangements, mitogenesis and anti-apoptotic (survival) activity. For example, LPA is released by activated platelets and accumulates in serum to low micromolar levels, where it is a prominent growth factor for many cell types. LPA has also been found in ascitic fluid from ovarian cancer patients where it promotes mitogenesis of ovarian tumor cells. Interestingly, the LPA found in serum vs. ascitic fluid differs in that LPA from ascitic fluid is reportedly enriched in 2-acyl LPA species. Study of this 2-acyl LPA isoform is made

difficult however by its chemical instability, i.e., the rapid migration of the acyl chain to the thermodynamically favored 1 position in an aqueous environment. Transient rises in blood pressure in rats and guinea pigs has also been documented following intravenous LPA injection. The induction of platelet aggregation and fibroblast recruitment along with its mitogenic capabilities implicate this lipid as a wound healing hormone.

LPA signals cells in part via a set of G protein-coupled receptors named LPA1, LPA2, and LPA3 (formerly Edg-2, Edg-4 and Edg-7). These receptors share 50-55% identical amino acids and cluster with five other receptors (S1P1, S1P2, S1P3, S1P4, S1P5 (formerly Edg-1, Edg-5, Edg-3, Edg-6, Edg-8) for the structurally-related lipid sphingosine 1-phosphate (S1P). LPA1 is most associated with activation of Giα pathways and is expressed in oligodendrocytes and peripheral tissues while LPA2 and LPA3 are associated most prominently with Gq/11α pathways. LPA2 mRNA is found in testis and peripheral blood leukocytes while LPA3 mRNA has been localized to prostate, testes, pancreas, kidney, and heart.

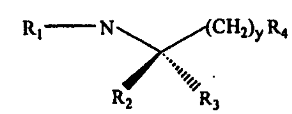
The physiologic implications of occupation of individual LPA receptors are largely unknown due in part to a lack of receptor type selective ligands. Therefore there is a need for compounds that have strong affinity and high selectivity for LPA receptors. The present invention is directed to a series of 2-substituted ethanolamide derivatives that vary in degrees of size, hydrophobicity, and stereochemistry. The parent compound of the claimed series, N-acyl ethanolamide phosphate (NAEPA) has been shown to be nearly indistinguishable from LPA in evoking platelet aggregation and GTP[γ ^{35}S] binding at LPA1 and LPA2 containing membranes but is distinctly less active than LPA at recombinant LPA3 or in depolarizing *Xenopus* oocytes.

Three 2-substituted NAEPA compounds have already been reported. A 2-carboxyl-containing compound (named 'NASPA' for N-palmitoyl serine phosphate) has been documented to antagonize both LPA-driven platelet aggregation (Sugiura *et al.*, 1994 ***Arch Biochem Biophys*** **311:** 358-368) and oocyte depolarization (Santos *et al.*, 2000 *NYAS Meeting Report;* Annals New York Academy of Sciences p 232-241) and is a partial agonist at mammalian LPA receptors. A 2-methylene hydroxy- containing compound, which is an analog of 2-acyl LPA wherein the ester is

replaced by an amide, has been reported as activating recombinant LPA receptors in a stereoselective fashion while mitogenic responses and platelet aggregation did not show this stereoselectivity. Finally, a third compound (named 'PNPA' for N-palmitoyl-norleucinol-1-phosphate) has n-butyl located at the 2 position. This compound aggregates human platelets without regard to stereoselectivity.

Summary of the Invention

Starting with an LPA analog wherein the glycerol is replaced with ethanolamine (N-acyl ethanolamide phosphate, N-acyl EPA) as a lead structure a series of new chemical entities with a variety of substitutions at the second carbon atom were synthesized and tested for activity at the LPA receptors. LPA analogs were prepared and found to have a range of activities including agonism, with various degrees of selectivity at individual LPA receptors, as well as compounds with antagonist activity at the LPA receptors. More particularly, the LPA analogs of the present invention include compounds with the general structure:



wherein R_1 is a large lipophilic group, R_2 and R_3 are various substituents, n is an integer from 1-10, and R_4 is selected from the group consisting of hydroxyl, phosphate, methylene phosphonate, α-substituted methylene phosphonate, phosphate anlogs and phosphonate analogs. Selective agonists and antagonists at LPA receptors will be useful therapeutically in a wide variety of human disorders.

Brief Description of the Drawings

Fig. 1 is a graphic representation of the calcium mobilization in A431 cells treated with 10 μM of each compound. Columns 1-13 represent the administration of VPC12086, VPC12101, VPC12109, VPC12115, VPC12098, VPC12105, VPC12084, VPC12255, VPC31144, VPC31143, VPC31180, VPC31139 and LPA, respectively. 100% calcium indicates the signal observed after permeabilization with digitonin. Bars are representative of three experiments.

Fig. 2A-2D are graphic representations of the effect of VPC12249 and VPC12204 on GTP[γ^{35}S] binding. GTP[γ^{35}S] binding assays of LPA1 (Fig. 2A), LPA2 (Fig. 2B), and LPA3 (Fig. 2C) transfected HEK293T cell membranes showing LPA concentration response curves with increasing concentrations of VPC12249. Fig. 2D shows the effect of VPC12204 (enantiomer of VPC 12249) on GTP[γ^{35}S] binding at LP3 transfected HEK293T cell membranes. Points are in triplicate and are representative of at least two experiments.

Fig. 3 illustrates dose-response curves for LPA, VPC12031 and VPC12060 stimulation of GTPγ[^{35}S] in Rh7777 of HEK293T membranes at LPA1, LPA2, and LPA3 receptors. Fig 3A represents stimulation of GTPγ[^{35}S] binding to LPA1 in Rh7777 membranes; Fig 3B represents stimulation of GTPγ[^{35}S] binding to LPA2 in HEK293T membranes; Fig 3C represents stimulation of GTPγ[^{35}S] binding to LPA3 in HEK293T membranes.

Fig. 4A and 4B illustrate the inhibitory activity of NOHPP (VPC12031) and alpha keto NOHPP analog (VPC12060), respectively, at the LPP1 (PAP2a) phosphatase.

Fig. 5A and 5B illustrate the inhibitory activity of NOHPP (VPC12031) and alpha keto NOHPP analog (VPC12060), respectively, at the LPP2 (PAP3a) phosphatase.

Fig. 6A and 6B illustrate the inhibitory activity of NOHPP (VPC12031) and alpha keto NOHPP analog (VPC12060), respectively, at the LPP3 (PAP2b) phosphatase.

Detailed Description of the Invention

Definitions

In describing and claiming the invention, the following terminology will be used in accordance with the definitions set forth below.

As used herein, the term "purified" and like terms relate to the isolation of a molecule or compound in a form that is substantially free of contaminants normally associated with the molecule or compound in a native or natural environment.

As used herein, the term "treating" includes prophylaxis of the specific disorder or condition, or alleviation of the symptoms associated with a specific disorder or condition and/or preventing or eliminating said symptoms.

As used herein, an "effective amount" means an amount sufficient to produce a selected effect. For example, an effective amount of an LPA receptor antagonist is an amount that decreases the cell signaling activity of the LPA receptor.

As used herein, the term "halogen" means Cl, Br, F, and I. Especially preferred halogens include Cl, Br, and F. The term "haloalkyl" as used herein refers to a C_1 -C_4 alkyl radical bearing at least one halogen substituent, for example, chloromethyl, fluoroethyl or trifluoromethyl and the like.

The term "C_1 -C_n alkyl" wherein n is an integer, as used herein, represents a branched or linear alkyl group having from one to the specified number of carbon atoms. Typically C_1 -C_6 alkyl groups include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, butyl, iso-butyl, sec-butyl, tert-butyl, pentyl, hexyl and the like.

The term "C_2 -C_n alkenyl" wherein n is an integer, as used herein, represents an olefinically unsaturated branched or linear group having from 2 to the specified number of carbon atoms and at least one double bond. Examples of such groups include, but are not limited to, 1-propenyl, 2-propenyl, 1,3-butadienyl, 1-butenyl, hexenyl, pentenyl, and the like.

The term "C_2 -C_n alkynyl" wherein n is an integer refers to an unsaturated branched or linear group having from 2 to the specified number of carbon atoms and at least one triple bond. Examples of such groups include, but are not limited to, 1-propynyl, 2-propynyl, 1-butynyl, 2-butynyl, 1-pentynyl, and the like.

The term "C_3 -C_n cycloalkyl" wherein n = 4-8, represents cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cyclooctyl.

As used herein, the term "optionally substituted" refers to from zero to four substituents, wherein the substituents are each independently selected. More preferredly, the term refers to from zero to three independently selected substituents. Each of the independently selected substituents may be the same or different than other substituents.

As used herein the term "aryl" refers to a mono- or bicyclic carbocyclic

ring system having one or two aromatic rings including, but not limited to, phenyl, benzyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl, and the like. Substituted aryl includes aryl compounds having one or two C_1 -C_6 alkyl, halo or amino substituents. The term (C_5-C_8 alkyl)aryl refers to any aryl group which is attached to the parent moiety via the alkyl group.

The term "heterocyclic group" refers to a C_3-C_8 cycloalkyl group containing from one to three heteroatoms wherein the heteroatoms are selected from the group consisting of oxygen, sulfur, and nitrogen.

The term "bicyclic" represents either an unsaturated or saturated stable 7- to 12-membered bridged or fused bicyclic carbon ring. The bicyclic ring may be attached at any carbon atom which affords a stable structure. The term includes, but is not limited to, naphthyl, dicyclohexyl, dicyclohexenyl, and the like.

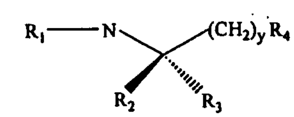
The terms 16:0, 18:0, 18:1, 20:4 or 22:6 hydrocarbon refers to a branched or straight alkyl or alkenyl group, wherein the first integer represents the total number of carbons in the group and the second integer represent the number of double bonds in the group.

The Invention

Lysophosphatidic acid (LPA) elicits a wide variety of responses from cells and tissues including calcium mobilization, changes in cell shape and motility, mitogenesis and anti-apoptosis. These effects are mediated by at least three LPA receptors (LPA1, LPA2 and LPA3) that have been cloned. Assignment of a physiological response to stimulation of a particular LPA receptor(s) is made difficult by lack of ligands that discriminate amongst receptor subtypes. The problem is exacerbated by the existence of at least three lyso-lipid phosphate phosphatases (LPPs) that act as ecto-phosphatases in degrading extra-cellular LPA as well as the potential for LPA to be acylated by LPA acyl transferases to yield another mediator, phosphatidic acid. Therefore, the discovery of new chemical entities that are (1) LPA receptor subtype selective agonists or antagonist and/or (2) LPA receptor agonists that are resistant to enzymatic degradation and/or (3) inhibitors of the LPPs is highly desirable.

A GTP[γ35 S] binding assay was developed to analyze directly the activation of individual LPA receptors, and thus allow the identification of LPA receptor agonists and antagonists as well as determine the relative efficacies and potencies at each receptor in a common system. The same results were obtained regardless of whether the recombinant receptor used exogenous G proteins (HEK293T cells) or endogenous G proteins (RH7777 cells) and further, the activities measured in the broken cell assay predicted the responses seen in whole cell assays. Thus the primary assay used in the present invention for compound potency and efficacy is a valid measure of activity at LPA/Edg receptors.

Starting with a LPA analog wherein the glycerol is replaced with ethanolamine (N-acyl ethanolamide phosphate, N-acyl EPA) as a lead structure a series of new chemical entities with a variety of substitutions at the second carbon atom were synthesized and tested for activity at the LPA receptors. In particular, both the relative potency and efficacy of the 2-substituted N-acyl EPA compounds were measured. The 2-substituted N-acyl EPA compounds of the present invention include compounds having the general structure:



I

wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl, C_8-C_{22} alkenyl, C_8-C_{22} alkanoyl, C_8-C_{22} alkenoyl,

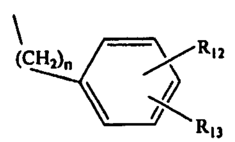
$$-\overset{\displaystyle O}{\overset{\|}{C}}(CH_2)_m-Z-R_{11} \quad \text{and} \quad -(CH_2)_m-Z-R_{11}\ ;$$

wherein m is 0-20;

Z is selected from the group consisting of C_3-C_{10} cycloalkyl, C_3-C_{15} bicycloalkyl, C_5-C_{10} heterocyclic and aryl;

R_{11} is selected from the group consisting of C_1-C_{10} alkyl, C_1-C_{20} alkoxyl, C_1-C_{20} alkylthio, and C_1-C_{20} alkylamino;

R_2 and R_3 are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, -(C_1-C_4 alkyl)OH, -(C_1-C_4 alkyl)NH_2, -$COOR_5$, -(C_1-C_4 alkyl)$COOR_5$, -(C_1-C_{10} alkyl)aryl, C_3-C_8 cycloalkyl, C_3-C_8 heterocyclic, C_7-C_{12} bicyclic, (C_5-C_8 alkyl)aryl, (C_5-C_8 alkenyl)aryl, (C_5-C_8 alkynyl)aryl, and



wherein n is 0-10;

R_5 is selected from the group consisting of H and C_1-C_4 alkyl;

R_{12} is selected from the group consisting of halo, C_1-C_{10} alkyl, (C_0-C_{12} alkyl)aryl, (C_2-C_{12} alkenyl)aryl, (C_2-C_{12} alkynyl)aryl, -(C_1-C_4 alkyl)OH, -(C_2-C_{12} alkenyl)OH, SR_6, SOR_6, NHR_6 and OR_6;

R_{13} is selected from the group consisting of H, halo, C_1-C_{10} alkyl, (C_0-C_{12} alkyl)aryl, (C_2-C_{12} alkenyl)aryl, (C_2-C_{12} alkynyl)aryl, -(C_1-C_4 alkyl)OH, -(C_2-C_{12} alkenyl)OH, SR_6, SOR_6, NHR_6 and OR_6;

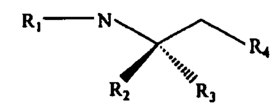
wherein R_6 is selected from the group consisting of C_1-C_{16} alkyl, C_2-C_{16} alkenyl, C_2-C_{16} alkynyl, -(C_1-C_4 alkyl)R_7, -(C_2-C_4 alkenyl)R_7, -(C_1-C_4 carboxy)R_7 and -(C_2-C_4 alkynyl)R_7; and

R_7 is selected from the group consisting of optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_3-C_8 heterocyclic, optionally substituted C_7-C_{12} bicyclic and optionally substituted C_5-C_8 cycloalkenyl and optionally substituted aryl, wherein the ring structures are substituted with one or more substituents selected from the group of C_1-C_4 alkyl, C_1-C_4 alkoxyl, halo, amino or hydroxy groups;

y is 1-4; and

R_4 is selected from the group consisting of hydroxyl, phosphate and methylene phosphonate, α-substituted methylene phosphonate, thiophosphate and phosphonate analogs.

In accordance with one embodiment the LPA analog has the general structure



wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl, C_8-C_{22} alkenyl,

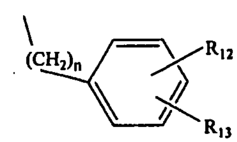
$$-\overset{\displaystyle O}{\overset{\|}{C}}(CH_2)_m-Z-R_{11} \quad \text{and} \quad -(CH_2)_m-Z-R_{11}\ ;$$

wherein m is 0-20;

Z is selected from the group consisting of C_3-C_{10} cycloalkyl, C_3-C_{15} bicycloalkyl, C_5-C_{10} heterocyclic and phenyl;

R_{11} is selected from the group consisting of C_1-C_{10} alkyl, C_1-C_{20} alkoxyl, C_1-C_{20} alkylthio, and C_1-C_{20} alkylamino;

R_2 and R_3 are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, -(C_1-C_4 alkyl)OH, -(C_1-C_4 alkyl)NH_2, -$COOR_5$, -(C_1-C_4 alkyl)$COOR_5$, -(C_1-C_{10} alkyl)aryl, C_3-C_8 cycloalkyl, C_3-C_8 heterocyclic, C_7-C_{12} bicyclic, (C_5-C_8 alkyl)aryl, (C_5-C_8 alkenyl)aryl, (C_5-C_8 alkynyl)aryl, and



wherein n is 0-10;

R_5 is selected from the group consisting of H and C_1-C_4 alkyl;

R_{12} is selected from the group consisting of halo, C_1-C_{10} alkyl, (C_0-C_{12} alkyl)aryl, (C_2-C_{12} alkenyl)aryl, (C_2-C_{12} alkynyl)aryl, -(C_1-C_4 alkyl)OH, -(C_2-C_{12} alkenyl)OH, SR_6, SOR_6, NHR_6 and OR_6;

R_{13} is selected from the group consisting of H, halo, C_1-C_{10} alkyl, (C_0-C_{12} alkyl)aryl, (C_2-C_{12} alkenyl)aryl, (C_2-C_{12} alkynyl)aryl, -(C_1-C_4 alkyl)OH, -(C_2-C_{12} alkenyl)OH, SR_6, SOR_6, NHR_6 and OR_6;

wherein R_6 is selected from the group consisting of C_1-C_{16} alkyl, C_2-C_{16} alkenyl, C_2-C_{16} alkynyl, -(C_1-C_4 alkyl)R_7, -(C_2-C_4 alkenyl)R_7, -(C_1-C_4 carboxy)R_7 and -(C_2-C_4 alkynyl)R_7; and

R_7 is selected from the group consisting of optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_3-C_8 heterocyclic, optionally substituted C_7-C_{12} bicyclic and optionally substituted aryl, wherein the ring structures are substituted with one or more substituents selected from the group of C_1-C_4 alkyl, C_1-C_4 alkoxyl, halo, amino or hydroxy groups; and

R_4 is selected from the group consisting of hydroxyl, phosphate, methylene phosphonate, α-substituted methylene phosphonate, thiophosphonate and phosphonate analogs. In one embodiment, R_1 is selected from the group consisting of

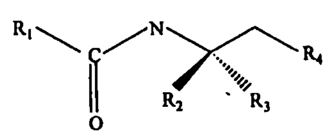
$$-\overset{\displaystyle O}{\overset{\|}{C}}(CH_2)_m-Z-R_{11} \quad \text{and} \quad -(CH_2)_m-Z-R_{11}\ ;$$

R_3 is H and R_4 is OPO_3^{-2} or methylene phosphonate. Alternatively, R_1 is selected from the group consisting of

$$-\overset{\displaystyle O}{\overset{\|}{C}}(CH_2)_m-Z-R_{11} \quad \text{and} \quad -(CH_2)_m-Z-R_{11}\ ;$$

R_2 is H and R_4 is OPO_3^{-2} or methylene phosphonate.

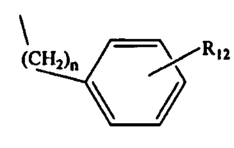
In another embodiment of the present invention the LPA analog is a compound represented by the structure:



II

wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl and C_8-C_{22} alkenyl, substituted C_8-C_{22} alkyl and substituted C_8-C_{22} alkenyl;

R_2 and R_3 are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, -(C_1-C_4 alkyl)OH, -(C_1-C_4 alkyl)NH_2, -$COOR_5$, -(C_1-C_4 alkyl)$COOR_5$, -(C_1-C_{10} alkyl)aryl, C_3-C_8 cycloalkyl, C_3-C_8 heterocyclic, C_7-C_{12} bicyclic, (C_5-C_8 alkyl)aryl, (C_5-C_8 alkenyl)aryl, (C_5-C_8 alkynyl)aryl, and



wherein n is 0-10;

R_5 is selected from the group consisting of H and C_1-C_4 alkyl;

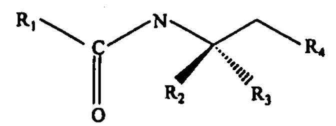
R_{12} is selected from the group consisting of halo, C_1-C_{10} alkyl, (C_0-C_{12} alkyl)aryl, (C_2-C_{12} alkenyl)aryl, (C_2-C_{12} alkynyl)aryl, -(C_1-C_4 alkyl)OH, -(C_2-C_{12} alkenyl)OH, SR_6, SOR_6, NHR_6 and OR_6;

wherein R_6 is selected from the group consisting of C_1-C_{16} alkyl, C_2-C_{16} alkenyl, C_2-C_{16} alkynyl, -(C_1-C_4 alkyl)R_7, -(C_2-C_4 alkenyl)R_7, -(C_1-C_4 carboxy)R_7 and -(C_2-C_4 alkynyl)R_7; and

R_7 is selected from the group consisting of optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_3-C_8 heterocyclic, optionally substituted C_7-C_{12} bicyclic and optionally substituted C_3-C_8 cycloalkenyl and optionally substituted aryl, wherein the ring structures are substituted with one or more substituents selected from the group of C_1-C_4 alkyl, C_1-C_4 alkoxyl, halo, amino or hydroxy groups; and

R_4 is selected from the group consisting of hydroxyl, phosphate, methylene phosphonate, α-substituted methylene phosphonate, thiophosphonate and phosphonate analogs.

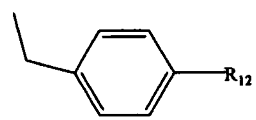
In accordance with one embodiment, the LPA analogs of the present invention are represented by the structure:



II

wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl, C_8-C_{22} alkenyl;

R_2 and R_3 are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, -(C_1-C_4 alkyl)OH, -(C_1-C_4 alkyl)NH_2, -$COOR_5$, -(C_1-C_4 alkyl)$COOR_5$, -(C_1-C_{10} alkyl)aryl, C_3-C_8 cycloalkyl, C_3-C_8 heterocyclic, C_7-C_{12} bicyclic, (C_5-C_8 alkyl)aryl, (C_5-C_8 alkenyl)aryl, (C_5-C_8 alkynyl)aryl, and



R_4 is selected from the group consisting of hydroxyl, phosphate, methylene phosphonate and α-substituted methylene phosphonate;

R_5 is selected from the group consisting of H and C_1-C_4 alkyl;

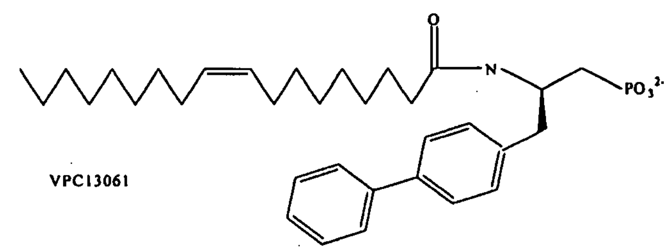
R_{12} is selected from the group consisting of halo, C_1-C_6 alkyl, C_1-C_6 alkyl(C_5-C_8 cycloalkenyl), C_1-C_6 alkenyl(C_5-C_8 cycloalkenyl), (C_0-C_{12} alkyl)aryl, (C_2-C_{12} alkenyl)aryl, (C_2-C_{12} alkynyl)aryl, -(C_1-C_4 alkyl)OH, -(C_1-C_4 alkenyl)OH, and OR_6;

R_6 is selected from the group consisting of C_1-C_{16} alkyl, C_2-C_{16} alkenyl, C_2-C_{16} alkynyl, -(C_1-C_4 carboxy)R_7, -(C_1-C_4 alkyl)R_7, -(C_2-C_4 alkenyl)R_7 and -(C_2-C_4 alkynyl)R_7; and

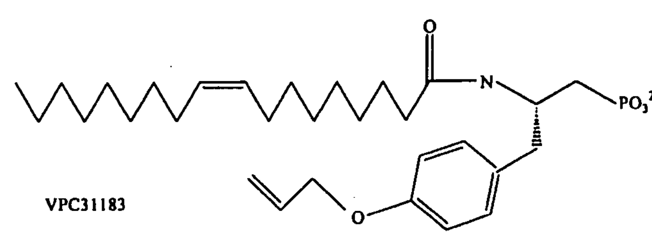
R_7 is selected from the group consisting of optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_3-C_8 heterocyclic, optionally substituted C_7-C_{12} bicyclic and optionally substituted C_5-C_8 aryl, wherein the ring structures are substituted with one or more substituents selected from the group of C_1-C_4 alkyl, C_1-C_4 alkoxyl, halo, amino or hydroxy groups. In one embodiment, R_1 is C_{13}-C_{17} alkyl or C_{17}-C_{21} alkenyl, and R_3 is H. More preferably R_1 is a 15:0, 17:0, 17:1, 19:4 or 21:6 hydrocarbon, R_3 is H and R_4 is OPO_3^{-2} or methylene phosphonate. The activities of

various members of this series have been tested at the three LPA receptor subtypes and found to have LPA receptor agonist and antagonist activities.

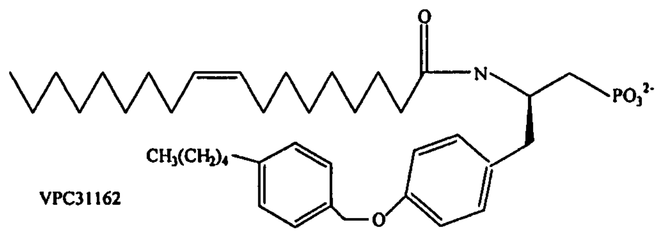
One embodiment of the present invention is directed to the compounds of Formula II wherein R_1 is a 15:0, 17:0, 17:1, 19:4 or 21:6 hydrocarbon, R_3 is H, R_4 is OPO_3^{-2} and R_2 is selected from the group consisting of 2-substitutions: methylene amino; para chloro benzyl; methylene benzyl; phenyl; methyl amino benzyl; aryl, and di-methyl. LPA analogs wherein R_4 is hydroxyl have not demonstrated activity as LPA receptor agonists or antagonists. However, it is anticipated that such compounds will be phosphorylated *in vivo* upon administration. Therefore compounds that have activity when R_4 is OPO_3^{-2} may be formulated as prodrugs by substituting a hydroxyl group for OPO_3^{-2} at R_4. In addition, the corresponding enantiomers are also encompassed by the present invention wherein R_1 is a 15:0, 17:0, 17:1, 19:4 or 21:6 hydrocarbon, R_2 is H, R_4 is OPO_3^{-2} and R_3 is selected from the group consisting of 2-substitutions: methylene amino; para chloro benzyl; methylene benzyl; phenyl; methyl amino benzyl; aryl, and di-methyl. Additional compounds encompassed by the present invention include:



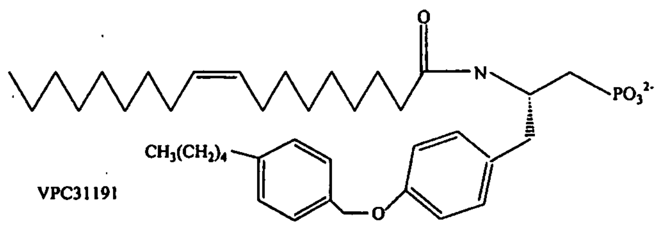
VPC13061



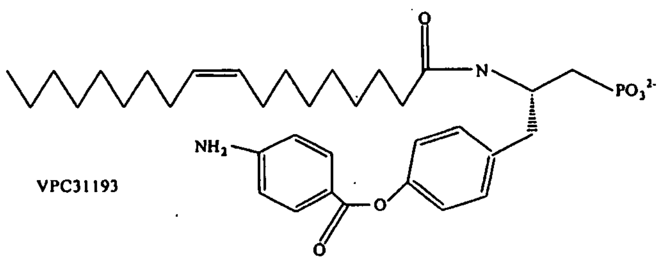
VPC31183



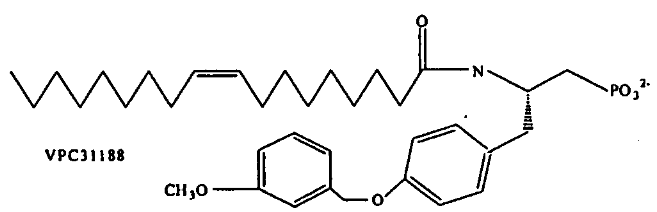
VPC31162



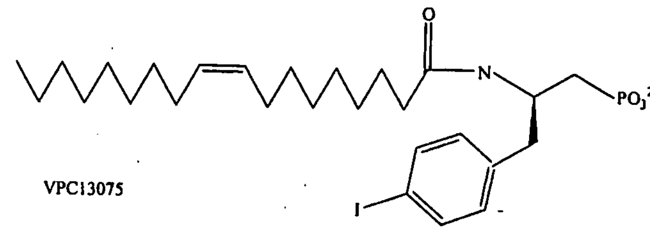
VPC31191



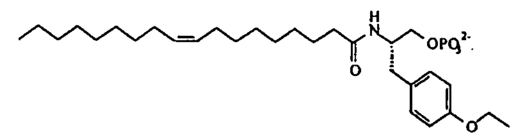
VPC31193



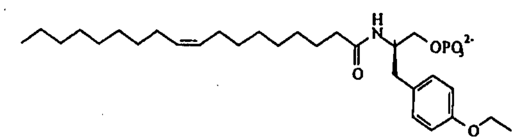
VPC31188



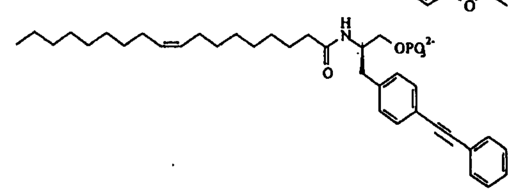
VPC13075



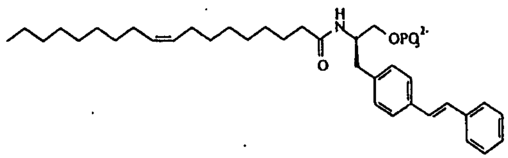
VPC31171



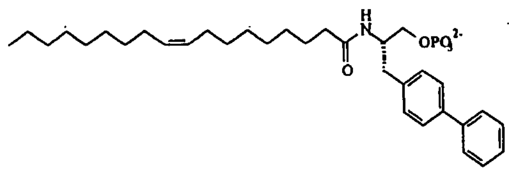
VPC31163



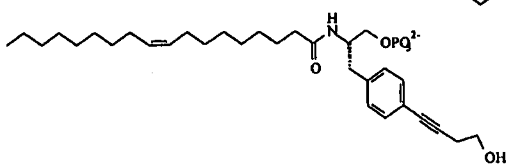
VPC13066



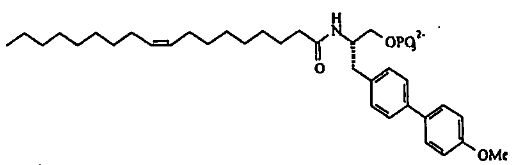
VPC13063



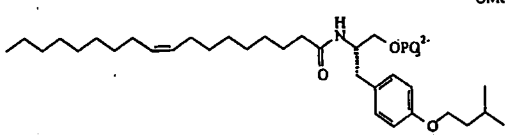
VPC13082



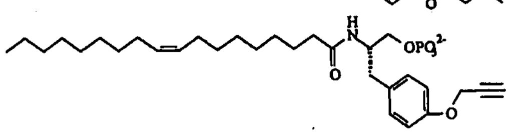
VPC13083



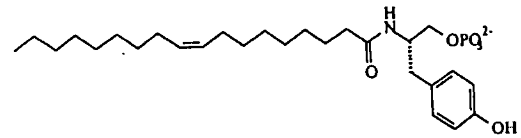
VPC13081

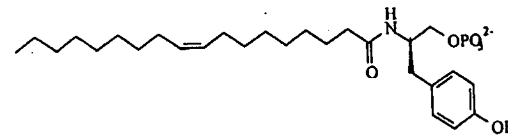


VPC31178

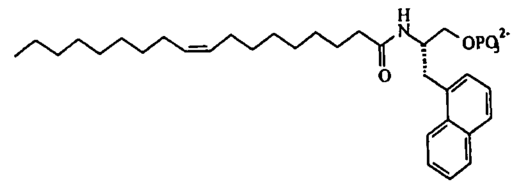


VPC31175

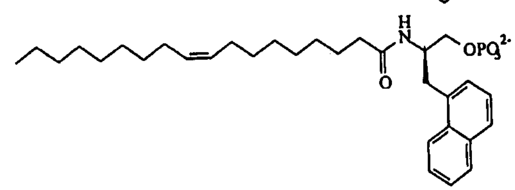




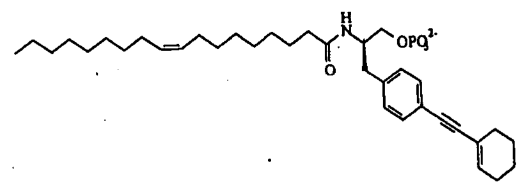
VPC12207



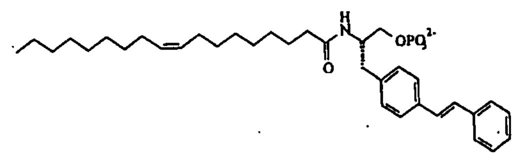
VPC12228



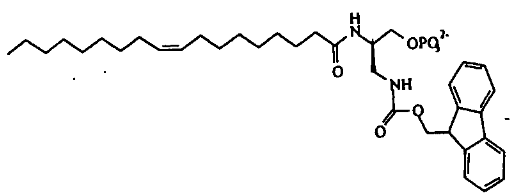
VPC12235



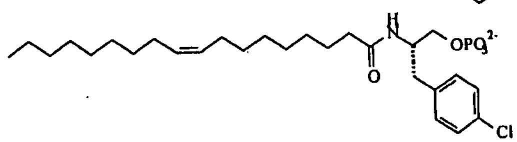
VPC13069

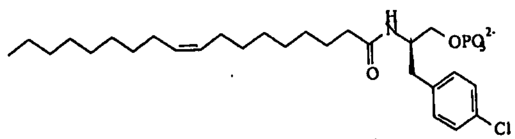


VPC13086

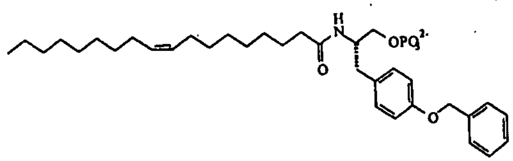


VPC12189

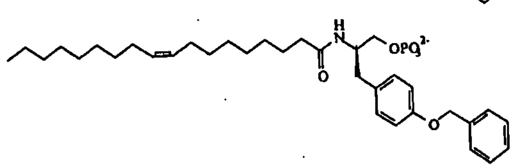




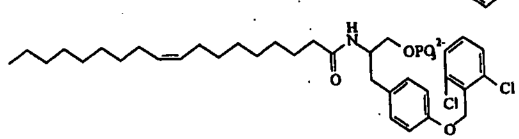
VPC12171



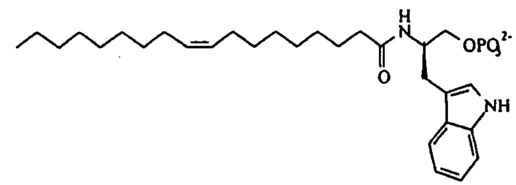
VPC12249



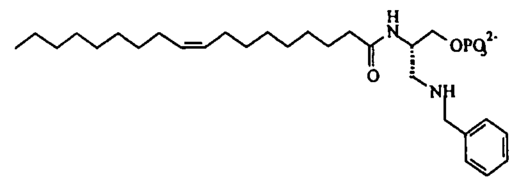
VPC12204

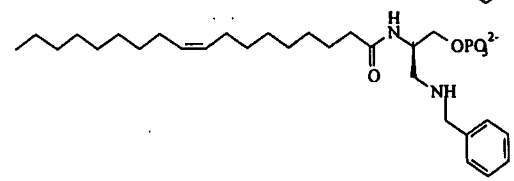


VPC12284

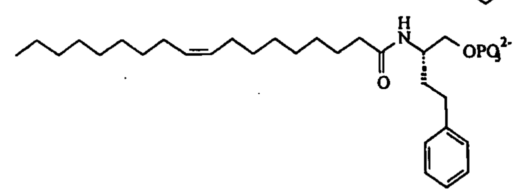


VPC12293

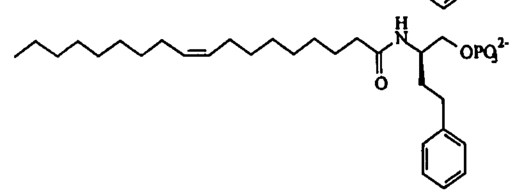




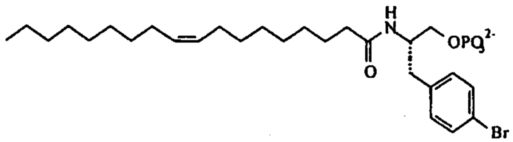
VPC12153



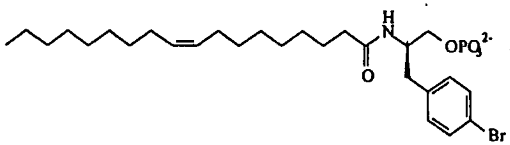
VPC12224



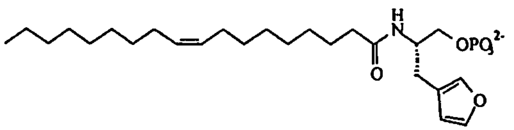
VPC12155



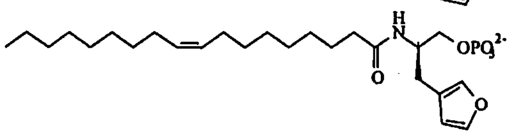
VPC12272



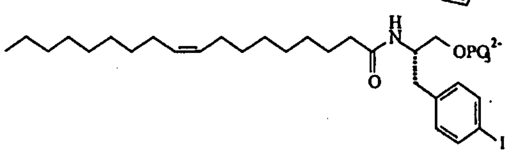
VPC12280

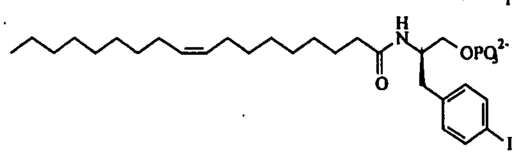


VPC12229

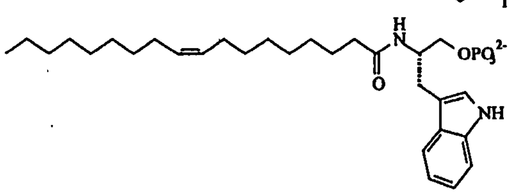


VPC12250

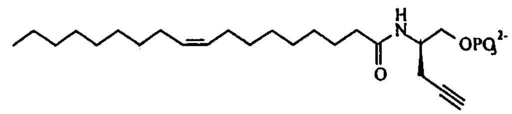




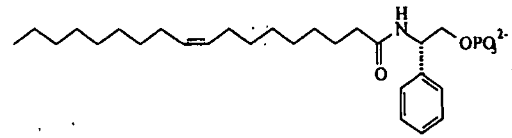
VPC13075



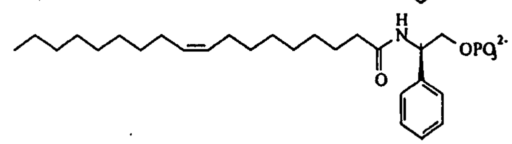
VPC12227



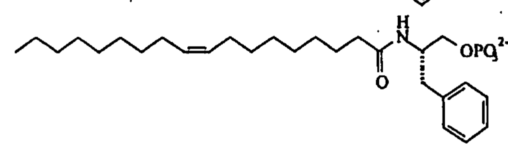
VPC12262



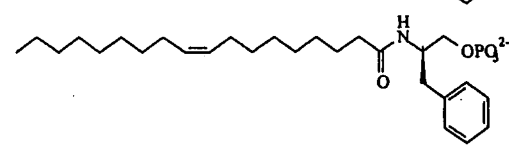
VPC12185



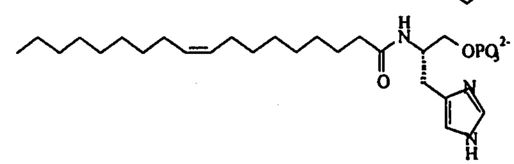
VPC12182



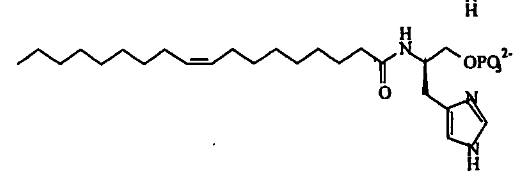
VPC12255

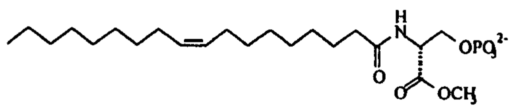


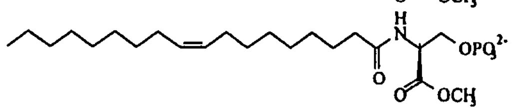
VPC12084

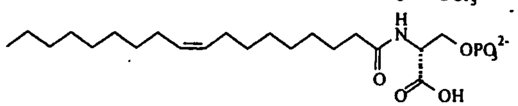


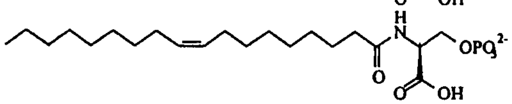
VPC30054

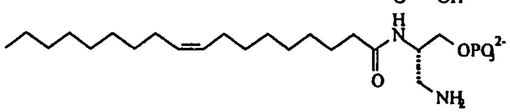


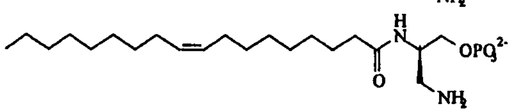

VPC31180

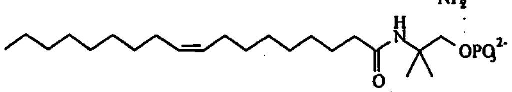

VPC31139

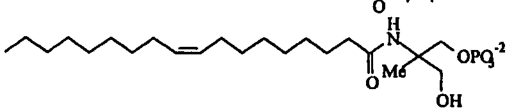

NASPA

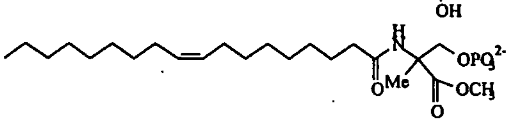

NASPA

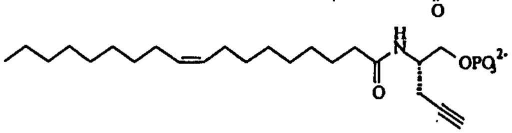

VPC12048


VPC12178

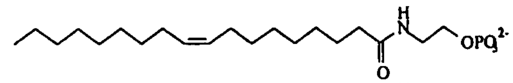

VPC12179

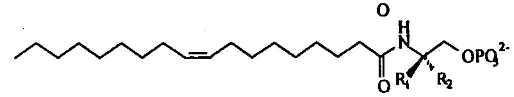

VPC13013 (rac)

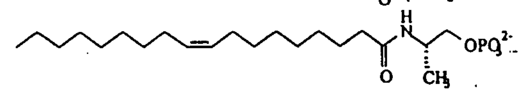

VPC12266 (rac)

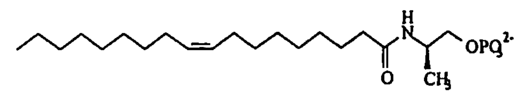

VPC12244

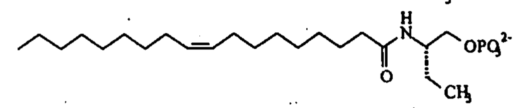
WO 02/29001 PCT/US01/30936

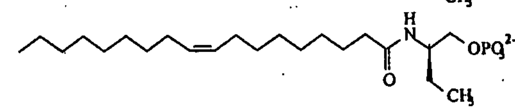

NOEPA

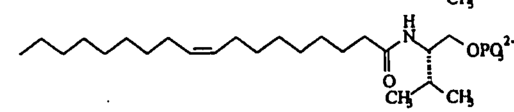

EPA

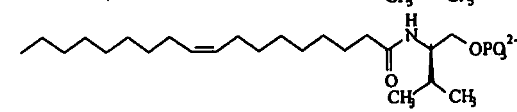

VPC12101

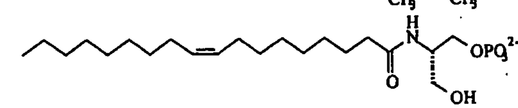

VPC12086

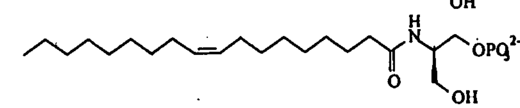

VPC12115


VPC12109


VPC40105

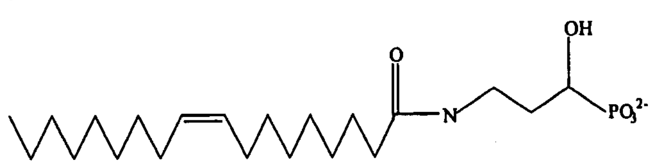

VPC12098


VPC31144


VPC31143

LPA is metabolized by a variety of conceivable routes including phosphatases, esterases and LPA acyl transferases or transported into cells. The LPA signal at receptors might be prolonged if the routes of degradation could be evaded or inhibited by LPA structural analogs. The LPA analogs of the present invention can be used, in accordance with one embodiment, to inhibit/evade endogenous LPA metabolic pathways including phosphotases, esterases, transporters and LPA acyl transferases. For example those LPA analogs of Formula I that lack and ester bond would be resistant to degradation by endogenous esterases. One embodiment of the present invention is directed to compounds that function as a LPA receptor agonists and antagonists that are resistant to hydrolysis by lyso-lipid phosphate phosphatases (LPPs) and are sub-type selective inhibitors of LPPs. Previously described LPA mimetics contain a phosphate group, and thus are likely susceptible to hydrolysis by LPPs. Furthermore, previously described LPA mimetics have not been shown to be selective for a particular LPA receptor.

Alpha hydroxy phosphonates are well known phosphate mimetics. For example, the compounds used clinically to treat osteoporosis (pamidronate, alendronate) are alpha hydroxy bisphosphonates that are analogs of pyrophosphate. LPA analogs were synthesized wherein the phosphate moiety is replaced by an alpha hydroxy phosphonate. Rather than being directly analogous to LPA, the LPP resistant compounds of the present invention are analogous to N-oleoyl ethanolamide phosphate – a compound that has been reported to be a potent, efficacious LPA mimetic. The structure of this compound, N-oleoyl-1-hydroxy propylamide phosphonic acid (NOHPP), is as follows:



The IUPAC name of NOHPP is (9Z)-N-(rac-3-hydroxy-3-phosphonopropyl)octadec-9-enamide.

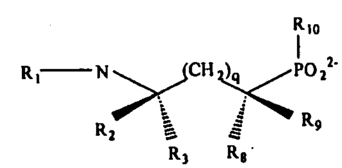
NOHPP inhibits LPP activity to varying degrees, and is a receptor subtype selective LPA mimetic. Specifically, NOHPP is fully efficacious (compared

to LPA) at the LPA1 and LPA2 receptors, but has little activity at the LPA3 receptor. NOHPP is about one log order less potent than 1-oleoyl LPA at both the LPA1 and LPA2 but greater than two log orders less potent at LPA3. Since the LPA receptor LPA3 has been reported to be less responsive to LPA with saturated acyl groups (Bandoh et al. (1999) J. Biol. Chem. vol. 274, pp. 27776-27785), NOHPP with a palmitoyl group (16:0) might have even greater selectivity for LPA1 and LPA2 vs. LPA3. NOHPP inhibits LPP activities with rank order potency LPP3 >LPP1>>LPP2. Furthermore, the LPA analogs of the present invention may function as LPP inhibitors even though they have poor agonist LPA activity. In accordance with one embodiment the LPP resistant compounds are used as selective inhibitors of LPP activity.

In addition, further derivatives of NOHPP are encompassed within the present invention wherein the amide linkage is replaced with a urea linkage, or an ethane or butane backbone is substituted for the propane backbone in NOHPP.

In accordance with one embodiment of the present invention a new series of compounds has been prepared that are analogous to N-oleoyl ethanolamide phosphate and are LPP resistant. These LPP resistant compounds have the general structure:

III



wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl, C_8-C_{22} alkenyl, C_8-C_{22} alkanoyl, C_8-C_{22} alkenoyl,

$$-\overset{O}{\overset{\|}{C}}(CH_2)_m-Z-R_{11} \quad \text{and} \quad -(CH_2)_m-Z-R_{11} \ ;$$

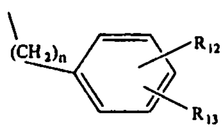
wherein m is 0-20;

Z is selected from the group consisting of C_3-C_{10} cycloalkyl, C_3-C_{15} bicycloalkyl, C_5-C_{10} heterocyclic and phenyl;

R_{11} is selected from the group consisting of C_1-C_{10} alkyl, C_1-C_{20}

alkoxyl, C_1-C_{20} alkylthio, and C_1-C_{20} alkylamino;

R_2 and R_3 are independently selected from the group consisting of H, hydroxy, C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, -(C_1-C_4 alkyl)OH, -(C_1-C_4 alkyl)NH_2, -$COOR_5$, -(C_1-C_4 alkyl)$COOR_5$, -(C_1-C_4 alkyl)aryl, C_3-C_8 cycloalkyl, C_3-C_8 heterocyclic, C_7-C_{12} bicyclic, (C_5-C_{10} alkyl)aryl, (C_5-C_8 alkenyl)aryl, (C_5-C_8 alkynyl)aryl, and



wherein n is 0-10;

R_5 is selected from the group consisting of H and C_1-C_4 alkyl;

R_{12} is selected from the group consisting of halo, C_1-C_{10} alkyl, (C_0-C_{12} alkyl)aryl, (C_2-C_{12} alkenyl)aryl, (C_2-C_{12} alkynyl)aryl, -(C_1-C_4 alkyl)OH, -(C_2-C_{12} alkenyl)OH, SR_6, SOR_6, NHR_6 and OR_6;

R_{13} is selected from the group consisting of H, halo, C_1-C_{10} alkyl, (C_0-C_{12} alkyl)aryl, (C_2-C_{12} alkenyl)aryl, (C_2-C_{12} alkynyl)aryl, -(C_1-C_4 alkyl)OH, -(C_2-C_{12} alkenyl)OH, SR_6, SOR_6, NHR_6 and OR_6;

wherein R_6 is selected from the group consisting of C_1-C_{16} alkyl, C_2-C_{16} alkenyl, C_2-C_{16} alkynyl, -(C_1-C_4 alkyl)R_7, -(C_2-C_4 alkenyl)R_7, -(C_1-C_4 carboxy)R_7 and -(C_2-C_4 alkynyl)R_7; and

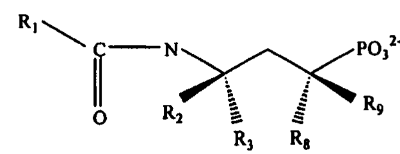
R_7 is selected from the group consisting of optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_3-C_8 heterocyclic, optionally substituted C_7-C_{12} bicyclic and optionally substituted C_5-C_8 cycloalkenyl and optionally substituted aryl, wherein the ring structures are substituted with one or more substituents selected from the group of C_1-C_4 alkyl, C_1-C_4 alkoxyl, halo, amino or hydroxy groups;

q is 1-4

R_8 and R_9 are are independently selected from H, hydroxyl, amino, COOH, halo, -PO_3; or R_8 and R_9 taken together form a keto group or a methylene group; and

R_{10} is selected from the group consisting of O, S and NH. In one embodiment, R_1 is C_8-C_{22} alkanoyl or C_8-C_{22} alkenoyl, R_2 is H, R_3 is hydroxy, C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, -(C_1-C_4 alkyl)OH, -(C_1-C_4 alkyl)NH_2, or -$COOR_5$ and q is 1.

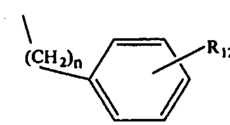
In one embodiment of the present invention the LPP resistant LPA analogs have the general structure:



V

wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl, C_8-C_{22} alkenyl, substituted C_8-C_{22} alkyl and substituted C_8-C_{22} alkenyl;

R_2 and R_3 are independently selected from the group consisting of H, hydroxy, C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, -(C_1-C_4 alkyl)OH, -(C_1-C_4 alkyl)NH_2, -$COOR_5$, -(C_1-C_4 alkyl)$COOR_5$, -(C_1-C_{10} alkyl)aryl and



wherein n is 1-10;

R_5 is selected from the group consisting of H and C_1-C_4 alkyl;

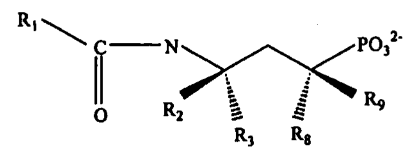
R_{12} is selected from the group consisting of halo, C_1-C_{10} alkyl, (C_0-C_{12} alkyl)aryl, (C_2-C_{12} alkenyl)aryl, (C_2-C_{12} alkynyl)aryl, -(C_1-C_4 alkyl)OH, -(C_2-C_{12} alkenyl)OH, SR_6, SOR_6, NHR_6 and OR_6;

wherein R_6 is selected from the group consisting of C_1-C_{16} alkyl, C_2-C_{16} alkenyl, C_2-C_{16} alkynyl, -(C_1-C_4 alkyl)R_7, -(C_2-C_4 alkenyl)R_7, -(C_1-C_4 carboxy)R_7 and -(C_2-C_4 alkynyl)R_7; and

R_7 is selected from the group consisting of optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_3-C_8 heterocyclic, optionally substituted C_7-C_{12} bicyclic and optionally substituted C_5-C_8 cycloalkenyl and optionally substituted aryl, wherein the ring structures are substituted with one or more substituents selected from the group of C_1-C_4 alkyl, C_1-C_4 alkoxyl, halo, amino or hydroxy groups; and

R_8 and R_9 are independently selected from H, hydroxyl, amino, COOH, halo, -PO_3; or R_8 and R_9 taken together form a keto group or a methylene group.

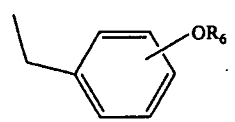
In one embodiment of the present invention the LPP resistant LPA analogs have the general structure:



IV

wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl, C_8-C_{22} alkenyl;

R_2 and R_3 are independently selected from the group consisting of H, hydroxy, C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, -(C_1-C_4 alkyl)OH, -(C_1-C_4 alkyl)NH_2, -$COOR_5$, -(C_1-C_4 alkyl)$COOR_5$, -(C_1-C_4 alkyl)aryl and



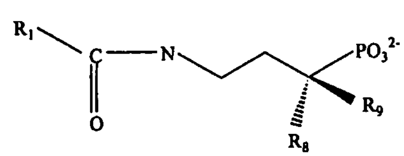
wherein R_5 is H or C_1-C_4 alkyl;

R_6 is selected from the group consisting of C_3-C_{16} alkyl, C_3-C_{16} alkenyl, -(C_1-C_4 alkyl)R_7;

R_7 is selected from the group consisting of C_3-C_8 cycloalkyl, C_3-C_8 heterocyclic, C_7-C_{12} bicyclic and C_5-C_8 aryl; and

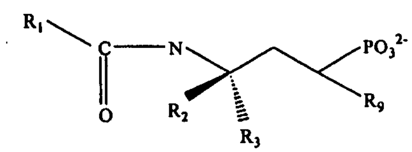
R_8 and R_9 are independently selected from the group consisting of H, hydroxyl, amino, COOH, halo, -PO_3; or R_8 and R_9 taken together form a keto group or a methylene group. In one embodiment, R_1 is C_{13}-C_{17} alkyl or C_{17}-C_{21} alkenyl, and more preferably R_1 is a 15:0, 17:0, 17:1, 19:4 or 21:6 hydrocarbon.

In one embodiment, the the LPP resistant LPA analog has the general structure



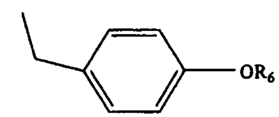
wherein R_1 is C_{13}-C_{17} alkyl or C_{17}-C_{21} alkenyl and R_8 and R_9 are independently selected from the group consisting of H, hydroxyl, fluoro, or R_8 and R_9 together form a keto group.

In another embodiment the compound has the general structure



wherein R_1 is C_{14}-C_{18} alkyl or C_{18}-C_{22} alkenyl;

R_2 and R_3 are independently selected from the group consisting of H, and

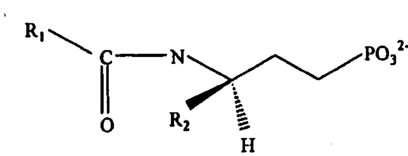


wherein R_6 is selected from the group consisting of C_3-C_{16} alkyl, C_3-C_{16} alkenyl, -(C_1-C_4 alkyl)R_7;

R_7 is selected from the group consisting of C_3-C_8 cycloalkyl, C_3-C_8 heterocyclic, C_7-C_{12} bicyclic, C_3-C_8 cycloalkenyl and aryl; and

R_9 is selected from the group consisting of H, hydroxyl, halo, keto and -PO_3.

In accordance with one embodiment compounds of the present invention have the general structure:

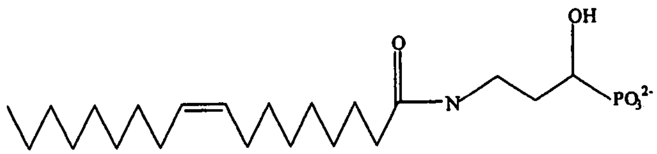


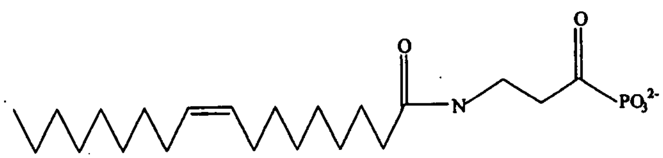
wherein R_1 is C_8-C_{22} alkyl or C_8-C_{22} alkenyl;

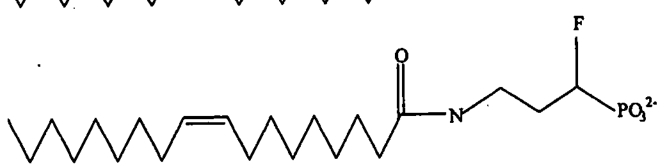
R_2 is selected from the group consisting of C_1-C_6 alkyl, -(C_1-C_4 alkyl)OH, -(C_1-C_4 alkyl)NH_2, $COOR_3$, -(C_1-C_4 alkyl)$COOR_3$, and -(C_1-C_4 alkyl)aryl; and

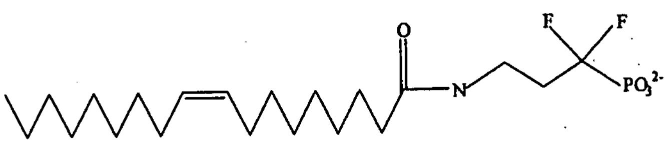
R_3 is selected from the group consisting of H and C_1-C_4 alkyl. In one preferred embodiment R_1 is C_{13}-C_{17} alkyl or C_{17}-C_{21} alkenyl, and R_2 is C_1-C_4 alkyl, methylene hydroxyl, carbomethyl, methylene amino or benzyl.

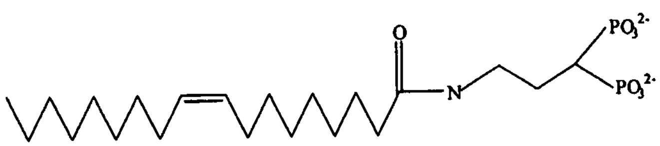
In one embodiment compounds that are based on the structure of NOHPP and fall within the scope of the present invention include the following compounds:











One aspect of the present invention is directed to the NOHPP analog, wls-b8L. The IUPAC name for wls-b8L is: **(9Z)-N-(3-phosphonopropyl)octadec-9-enamide**, and the trivial name is oleoylaminopropylphosphonate. The structure of wls-b8L is shown below:

wls-b8L is expected to be resistant to hydrolysis by phosphohydrolases by virtue of its containing a phosphonate, rather than a phosphate, group. Furthermore this chemical entity (wls-b8L) has two additional desirable properties. Firstly, wls-b8L is distinctly more selective regarding its agonist activity at a single LPA receptor (LPA1) than VPC12031 (the alpha hydroxy analog of NOHPP) and VPC12060 (the alpha keto analog of NOHPP) and, secondly, at concentrations up to 10 micromolar, wls-b8L does not elicit aggregation of human platelets.

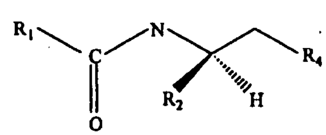
The novel LPA receptor agonists disclosed in the present invention are anticipated to have utility in a variety of clinical settings including but not limited to the acceleration of wound healing (including corneal wounds), the promotion of myelination (oligodendrocyte cell function) and for immuno-modulation. In particular, LPA has been reported (Balazs et al. *Am J Physiol Regul Integr Comp Physiol*, 2001 280(2):R466-472) as having activity in accelerating wound closing and increasing neoepithelial thickness. Accordingly, one embodiment of the present invention comprises administering a pharmaceutical composition comprising one or more of the LPA receptor agonists of the present invention to a mammalian species (including humans) to treat a wound, improve neuronal function or enhance an immune response of that species. LPA has been demonstrated to induce a modest dose-dependent

increase in proliferating cells, and a marked increase in the immigration of histiocyte-macrophage cells. Accordingly, in one embodiment compositions comprising an LPA receptor agonist is used to treat wounds, including burns, cuts, lacerations, surgical incisions, bed sores, and slow-healing ulcers such as those seen in diabetics. In one preferred embodiment a composition comprising an LPA agonist is administered to a patient to enhance wound repair. Typically the composition is administered locally as a topical formulation, however other standard routes of administration are also acceptable.

The investigation of various 2-substitutions revealed several trends (see Example 2, Table 1) in a series of compounds having the general structure of Formula I, wherein R_1 is a 17:1 hydrocarbon and R_4 is OPO_3^{-2}. First, each LPA receptor showed a marked (one log order or more) preference for one enantiomer. Second, most substitutions were well tolerated in that they resulted in agonist ligands. Third, the LPA3 receptor, unlike LPA1 and LPA2, differentiates between unsaturated and saturated acyl groups and that LPA3 appears to have a lower affinity for LPA and LPA analogs.

Although most active compounds were partial agonists with reduced potency (relative to LPA), the R-methyl compound (VPC12086) is notable in that it is more potent and efficacious than LPA at LPA_1. A pattern observed with all three LPA receptors was that the R configuration was more potent with all substituents, an exception is VPC31139, which is more active in the S configuration. These data indicate that each receptor has a single spatial region within the ligand binding site.

In accordance with one embodiment an LPA receptor agonist is provided having the general structure



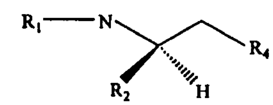
wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl, C_8-C_{22} alkenyl, substituted C_8-C_{22} alkyl and substituted C_8-C_{22} alkenyl;

R_2 is selected from the group consisting of C_1-C_4 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, -(C_1-C_4 alkyl)OH, -(C_1-C_4 alkyl)NH_2, -(C_1-C_4 alkyl)$COOR_5$, and -(C_0-C_{10} alkyl)aryl;

R_4 is selected from the group consisting of hydroxyl, phosphate, thiophosphate, methylene phosphonate and α substituted methylene phosphonate; and

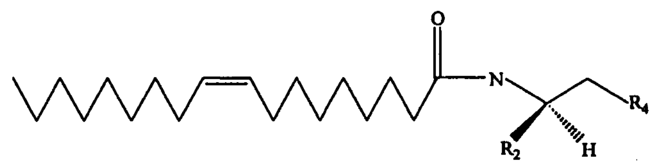
R_5 is selected from the group consisting of H and C_1-C_4 alkyl.

In one embodiment the compound has the general structure:



wherein R_1 is a saturated or unsaturated, substituted or non-substituted, straight or branched chain alkyl of about 8 to 22 carbon atoms where the #1 carbon may be in the form of a carbonyl group (C=O), i.e. the alkyl chain is joined by an amide linkage. In certain embodiments, R_1 is oleic acid (18:1) or palmitic acid (16:0) combined in an amide linkage; R_2 is methyl, ethyl, propyl, isopropyl, butyl, methylene hydroxy, methylene amino, methylene alkyne, phenyl, benzyl, methylene furan, methylene-2-naphthalene; and R_4 is hydroxyl (OH), phosphate (PO_4), or methylene phosphonate (CH_2PO_3). When R_4 is methylene phosphonate, the carbon alpha to the phosphorus can be optionally substituted with an hydroxyl, keto, fluoro or phosphonate moieties or di-substituted with fluoro or hydroxy and phosphonate substituents.

In accordance with one embodiment the LPA receptor agonist is provided having the general structure:



wherein R_2 is methyl, ethyl, propyl, isopropyl, butyl, methylene hydroxy, methylene amino, methylene alkyne, phenyl, benzyl, methylene furan, methylene-2-naphthalene; and R_4 is hydroxyl (OH), phosphate (PO_4), or methylene phosphonate (CH_2PO_3).

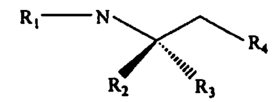
In accordance with one embodiment of the present invention LPA receptor subtype-selective compounds having agonist and/or antagonist properties can be administered to a subject to treat or prevent a disorder of abnormal cell growth and differentiation. These disorders include, but are not limited to, Alzheimer's disease, aberrant corpus luteum formation, osteoarthritis, osteoporosis, anovulation, Parkinson's disease, multiple sclerosis and rheumatoid arthritis.

As noted above LPA3 has a lower affinity for LPA and LPA analogs. It is believed that LPA3 may play a role in feedback inhibition of activity at LPA1 and LPA2. Therefore it is anticipated that an LPA3 subtype-selective agonist can be used to decrease LPA1 and LPA2 mediated activities. Thus in accordance with one embodiment an LPA3 subtype-selective agonist can be administered to a subject to treat or prevent a disorder of abnormal cell growth and differentiation, including cancer. To be considered a subtype selective agonist/antagonist, the compound mus be 10x more potent, and more preferably 100x more potent, at the preferred LPA receptor. For example, it appears that mono-unsaturated substituents at R_1 of the LPA receptor analogs of Formula I are significantly more potent (equipotent to LPA) than the saturated compound at LPA3. LPA1 and LPA2 do not exhibit this selectivity for unsaturated vs. saturated side chains.

Similarly LPA receptor antagonist can also be used to inhibit LPA mediated activities and thus treat disorders of abnormal cell growth and differentiation as well as inflammatory diseases. These disorders include, but are not limited to, Alzheimer's disease, aberrant corpus luteum formation, osteoarthritis, osteoporosis, anovulation, Parkinson's disease, multiple sclerosis, rheumatoid arthritis and treatment

of cancer. Such antagonists can then be formulated as pharmaceutical compositions using standard pharmaceutically acceptable carriers, fillers, solublizing agents and stabilizers known to those skilled in the art. Pharmaceutical compositions comprising the LPA receptor subtype-selective agonist and/or antagonist are administered to an individual in need thereof by any number of routes including, but not limited to, topical, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In accordance with one embodiment LPA receptor antagonists are provided, wherein the antagonists have the general structure



wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl, C_8-C_{22} alkenyl, C_8-C_{22} alkanoyl, C_8-C_{22} alkenoyl,

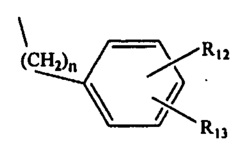
$$-\overset{O}{\overset{\|}{C}}(CH_2)_m-Z-R_{11} \quad \text{and} \quad -(CH_2)_m-Z-R_{11} \ ;$$

wherein m is 0-20;

Z is selected from the group consisting of C_3-C_{10} cycloalkyl, C_3-C_{15} bicycloalkyl, C_5-C_{10} heterocyclic and phenyl;

R_{11} is selected from the group consisting of C_1-C_{10} alkyl, C_1-C_{20} alkoxyl, C_1-C_{20} alkylthio, and C_1-C_{20} alkylamino;

R_2 and R_3 are independently selected from the group consisting of H, and



wherein n is 0-10;

R_{12} is selected from the group consisting of C_1-C_{10} alkyl, (C_0-C_{12} alkyl)aryl, (C_2-C_{12} alkenyl)aryl, (C_2-C_{12} alkynyl)aryl, -(C_1-C_4 alkyl)OH, -(C_2-C_{12} alkenyl)OH, SR_6, SOR_6, NHR_6 and OR_6;

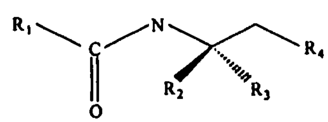
R_{13} is selected from the group consisting of H, C_1-C_{10} alkyl, (C_0-C_{12} alkyl)aryl, (C_2-C_{12} alkenyl)aryl, (C_2-C_{12} alkynyl)aryl, -(C_1-C_4 alkyl)OH, -(C_2-C_{12} alkenyl)OH, phenyl-4-methoxy, SR_6, SOR_6, NHR_6 and OR_6;

wherein R_6 is selected from the group consisting of C_1-C_{16} alkyl, C_2-C_{16} alkenyl, C_2-C_{16} alkynyl, -(C_1-C_4 alkyl)R_7, -(C_2-C_4 alkenyl)R_7, -(C_1-C_4 carboxy)R_7 and -(C_2-C_4 alkynyl)R_7;

R_7 is selected from the group consisting of optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_3-C_8 heterocyclic, optionally substituted C_7-C_{12} bicyclic, optionally substituted C_3-C_8 cycloalkenyl and optionally substituted aryl; and

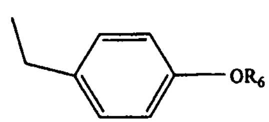
R_4 is selected from the group consisting of hydroxyl, phosphate, methylene phosphonate, α-substituted methylene phosphonate, thiophosphonate and phosphonate analogs. In one preferred embodiment n is 1, R_{13} is H and R_{12} is SR_6, SOR_6, NHR_6 or OR_6.

In accordance with one embodiment LPA receptor antagonists are provided, wherein the antagonists have the general structure



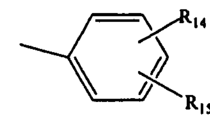
wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl, C_8-C_{22} alkenyl, substituted C_8-C_{22} alkyl and substituted C_8-C_{22} alkenyl;

R_2 and R_3 are independently selected from the group consisting of H, and



wherein R_6 is selected from the group consisting of C_3-C_{16} alkyl, C_3-C_{16} alkenyl, -(C_1-C_4 alkenyl)R_7, -(C_2-C_4 alkynyl)R_7 and -(C_2-C_4 alkyl)R_7;

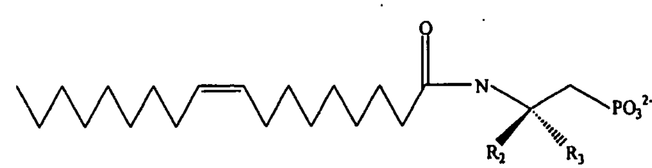
R_7 is



wherein R_{14} and R_{15} are independently selected from the group consisting of H, C_1-C_{12} alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, halo; and

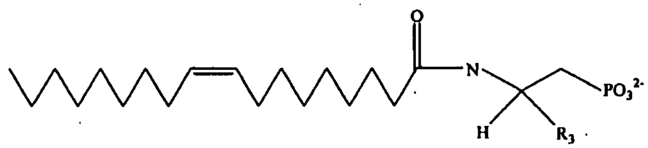
R_4 is selected from the group consisting of hydroxyl, phosphate, methylene phosphonate, with the proviso that R_2 and R_3 are not both H. In one embodiment, R_1 is C_{13}-C_{17} alkyl or C_{17}-C_{21} alkenyl and R_4 is phosphate or methylene phosphonate. More preferably R_1 is a 15:0, 17:0, 17:1, 19:4 or 21:6 hydrocarbon and R_4 is phosphate.

In accordance with one embodiment the LPA receptor antagonist has the general structure:

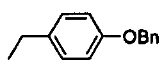
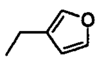
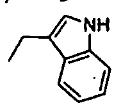
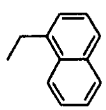
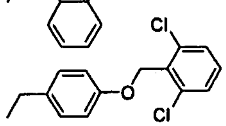
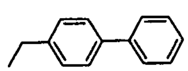
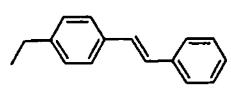
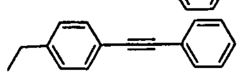
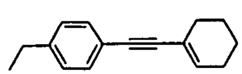


wherein R_2 and R_3 are independently selected from the group consisting of H, benzyl, methylene furan, methylene-2-naphthalene, methylene phenyl-4-O-benzyl, methylene phenyl-4-benzyl, methylene phenyl-4-chloro, methylene phenyl-4-*trans*-styrene, methylene phenyl-4-*cis*-styrene, methylene phenyl-4-O-2,6-dichlorobenzyl and methylene phenyl-4-phenyl. In one preferred embodiment R_3 is H.

Additional compounds that serve as antagonists of LPA receptor function include compounds of the general formula:



wherein R_3 is selected from the group:

	Compound name and Stereochemistry (R)	(S)
	VPC12204	VPC12249
	VPC12250	VPC12229
	VPC12193	VPC12227
	VPC12235	VPC12228
	VPC12284	
	VPC13061	VPC13082
	VPC13063	VPC13086
	VPC13066	
	VPC13069	VPC13089

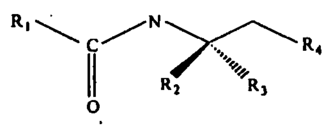
One example of an LPA antagonist in accordance with the present invention is VPC12249. That compound, having the backbone structure of Formula II, wherein R_1 is a 17:1 hydrocarbon, R_2 is H, R_4 is OPO_3^{-2} and R_3 contains the benzyl-4-oxybenzyl functionality in the S-configuration, which confers antagonistic activity at LPA1 and LPA3 receptors. VPC12249, to our knowledge, is the first specific LPA1/LPA3 receptor antagonist. This compound possesses high affinity for the LPA1 (ca. K_I = 130 nM) and LPA3 (ca. K_I = 425 nM) receptors. In addition compound VPC31162 (structure of Formula II, wherein R_1 is a 17:1 hydrocarbon, R_2 is H, R_4 is OPO_3^{-2} and R_3 is benzyl-4-oxybenzyl-4-pentane) has antagonist activity at the LPA2 receptor, but is an agonist at LPA1 and LPA3.

In accordance with one embodiment of the present invention the LPA analogs that exhibit LPA receptor antagonist activity can be used to treat ischemia reperfusion type injury. Interference with the supply of oxygenated blood to tissues is defined as ischemia. The effects of ischemia are known to be progressive, such that over time cellular vitality continues to deteriorate and tissues become necrotic. Total persistent ischemia, with limited oxygen perfusion of tissues, results in cell death and eventually in coagulation-induced necrosis despite reperfusion with arterial blood. A substantial body of evidence indicates that a significant proportion of the injury associated with ischemia is a consequence of the events associated with reperfusion of ischemic tissues, hence the term reperfusion injury.

To place reperfusion injury into a clinical perspective, there are three different degrees of cell injury, depending on the duration of ischemia: (1) With short periods of ischemia, reperfusion (and resupply of oxygen) completely restores the structural and functional integrity of the cell. Whatever degree of injury the cells have incurred can be completely reversed upon reoxygenation. (2) With longer periods of ischemia, reperfusion is not associated with the restoration of cell structure and function, but rather with deterioration and death of cells. The response to reoxygenation in this case is rapid and intense inflammation. (3) Lethal cell injury may develop during prolonged periods of ischemia, where reperfusion is not a factor. The reversibility of cell injury as a consequence of ischemia is determined not only by the type and duration of the injury, but also by the cell target. Neurons exhibit very high sensitivity to ischemia, whereas myocardial, pulmonary, hepatic and renal tissues

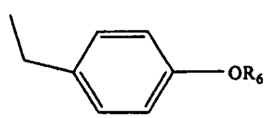
are intermediate in sensitivity. Fibroblasts, epidermis and skeletal muscle have the lowest susceptibility to ischemic injury, requiring several hours without blood supply to develop irreversible damage.

In accordance with the present invention a compound having the general structure:



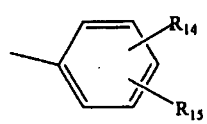
wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl, C_8-C_{22} alkenyl, substituted C_8-C_{22} alkyl and substituted C_8-C_{22} alkenyl;

R_2 and R_3 are independently selected from the group consisting of H, and



wherein R_6 is selected from the group consisting of C_3-C_{16} alkyl, C_3-C_{16} alkenyl, -(C_1-C_4 alkenyl)R_7, -(C_2-C_4 alkynyl)R_7 and -(C_2-C_4 alkyl)R_7;

R_7 is



wherein R_{14} and R_{15} are independently selected from the group consisting of H, C_1-C_{12} alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, halo; and

R_4 is selected from the group consisting of hydroxyl, phosphate, methylene phosphonate, with the proviso that R_2 and R_3 are not both H is administered to reduce of prevent reperfusion injury. In one embodiment, R_1 is C_{13}-C_{17} alkyl or C_{17}-C_{21} alkenyl and R_4 is phosphate or methylene phosphonate. More preferably R_1 is a 15:0, 17:0, 17:1, 19:4 or 21:6 hydrocarbon and R_4 is phosphate. In one preferred embodiment the compound is VPC12249.

The LPA receptor antagonist of the present invention can be formulated with pharmaceutically acceptable carriers, diluents, and solubilizing agents for administration to patient in need of such therapy. The compounds are preferably administered intravenously, but any standard route of administration is acceptable including oral delivery. In particular, if the LPA receptor antagonist is administered prior to tissue injury, such as to a patient prior to surgery, the LPA receptor antagonist can be administered orally.

Preferably, a therapeutically effective amount of the LPA receptor antagonist is administered intravenously in a physiologically acceptable carrier as early as possible, and most preferably within four hours of a reperfusion injury. Subsequent doses of the LPA receptor antagonist, can be administered intravenously or orally.

Example 1

Synthesis of 2-substituted, ethanolamide-based LPA Analogs

Chemicals for syntheses were purchased from Aldrich Chemical Company, Inc., Sigma Chemical Company, Inc., Advanced ChemTech Chemical Company, Inc. and/or NovaBiochem Chemical Company, Inc., and were used without further purification. The general approach used to synthesize the LPA analogs of the present invention is shown in Scheme 1. Briefly the seven steps are as follows:

General procedure A: Installation of Chiral Auxillary

To a solution of carbobenzyloxy-protected amino acid in diethyl ether at –78 °C is added triethylamine followed by pivaloyl chloride. The resulting thick white precipitate is stirred for 1 hr at 0 °C and then re-cooled to –78 °C. In a separate flask, a solution of [4S]-4-benzyl-2-oxaxolidinone in tetrahydrofuran (THF) is prepared. On cooling this solution to –78 °C, n-butyllithium is added *via* a syringe over 5 minutes. The resulting solution is cannulated to the flask containing the mixed anhydride. The mixture is stirred for 15 min at –78 °C and 30 min at 0 °C before quenching by addition of aqueous ammonium chloride. The resulting solution is concentrated and diluted with methylene chloride, extracted (3x) with sodium bicarbonate and brine and dried (Na_2SO_4). The imide product is isolated by flash chromatography.

General procedure B: Alpha-carbon Alkylation

To a solution of imide in dichloromethane at 0 °C is added titanium tetrachloride. After stirring for 5 minutes, diisopropylethylamine is added. The solution is stirred for 3 hours at 0 °C, and then the alkylbromide is added. The resulting solution is stirred for an additional 2 hours before cannulated to a vigorously stirring mixture of saturated aqueous sodium bicarbonate. The layers are separated and extracted (three times) with dichloromethane, dried (Na_2SO_4) and concentrated. The α,α-substituted product is isolated by flash chromatography.

General procedure C: Chiral Auxilliary Removal

To a solution of α,α-substituted amino acid in THF at 0 °C is added methanol and lithium borohydride. The solution is stirred for 1 hour and quenched by the addition of 1.0M sodium potassium tartrate and stirring for 10 min at 0 °C. The layers are separated and extracted (3x) with dichloromethane, dried (Na_2SO_4) and concentrated. The alcohol product is isolated by flash chromatography.

General procedure D: Phosphorylation

To a solution of alcohol in 1:1 THF/CH_2Cl_2 in an aluminum foil covered round bottom flask is added tetrazole. After stirring for 15 minutes, di-t-butyldiisoproylphosphoramidite is added. The solution is stirred for 6 hours and quenched with sodium metabisulfite at 0 °C, and extracted with ethylacetate. The organic layers are combined, dried (Na_2SO_4) and concentrated. The product is purified by flash chromatography.

General procedure E: N-Cbz Deprotection

To a solution of Cbz-protected phosphate in ethanol is added 10%Pd/C. The resulting solution is placed under H_2 atmosphere. After 2 hours, the solution is filtered and concentrated to provide the product.

General Procedure F: Addition of Fatty Acid Moiety

To a solution of amine in dichloromethane is added N,N-diisopropylethylamine. After stirring for 10 minutes, oleoyl chloride is added via a

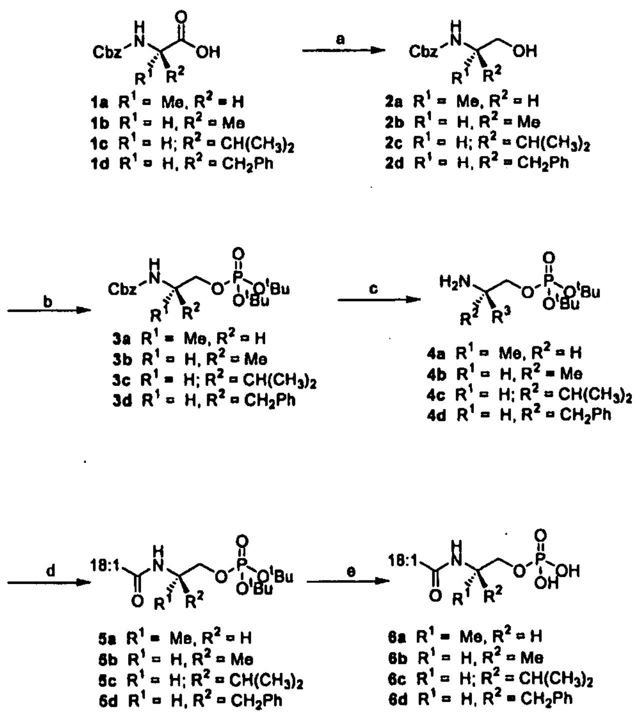
syringe over 20 minutes. The resulting solution is quenched by adding saturated aqueous ammonium chloride after 1 hour. The aqueous layer is extracted (3x) with ethyl acetate and the combined organic layers are dried (Na_2SO_4) and concentrated. The product is purified by flash chromatography.

General procedure G: Phosphate Deprotection

To a solution of protected phosphate in dichloromethane is added trifluoroacetic acid. After 2 hours, the solvent is removed via rotary evaporation and ether is added and removed. This process is repeated until the all trifluoroacetic acid is removed, providing the phosphate deprotected product.

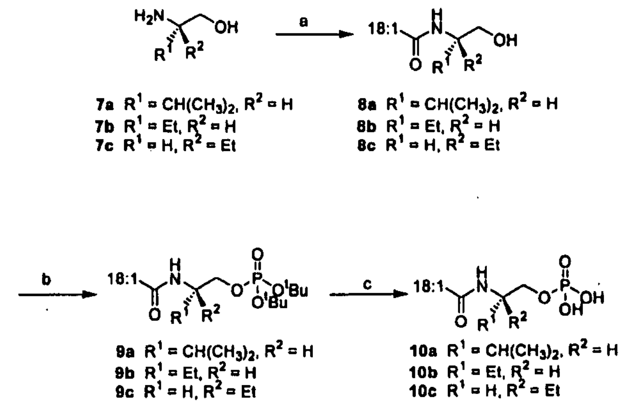
Additional synthetic details are provided in the following:

Scheme 1



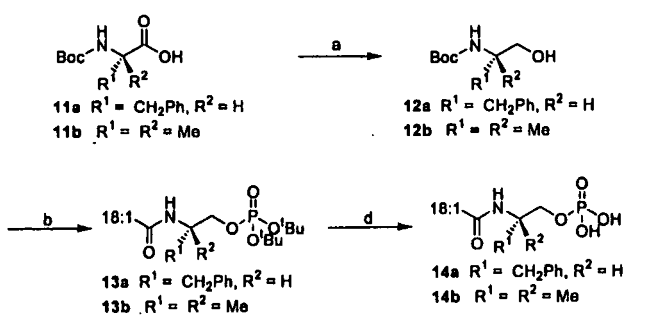
Reagents: (a) i. DIEA, isobutylchloroformate, ii. $NaBH_4$, H_2O; (b) 1H-Tetrazole, N,N-di-t-butyl diisopropylphosphoramidite; (c) 10% Pd/C, H_2; (d) Oleoyl chloride, DIEA or pyridine; (e) 1:1 TFA/CH_2Cl_2

Scheme 2



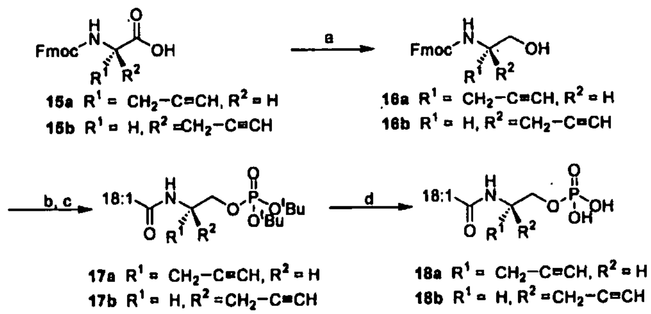
Reagents: (a) Oleoyl chloride, DIEA or pyridine; (b) 1H-Tetrazole, N,N-di-t-butyl diisopropylphosphoramidite; (c) 1:1 TFA/CH_2Cl_2

Scheme 3



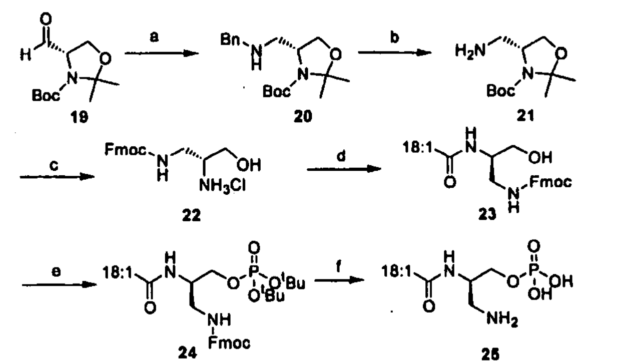
Reagents: (a) i. DIEA, isobutylchloroformate, ii. $NaBH_4$, H_2O; (b) i. 1:1 TFA/CH_2Cl_2; ii. Oleoyl chloride, DIEA or pyridine; (c) 1H-Tetrazole, N,N-di-t-butyl diisopropylphosphoramidite; (d) 10% Pd/C, H_2; (e) 1:1 TFA/CH_2Cl_2

Scheme 4



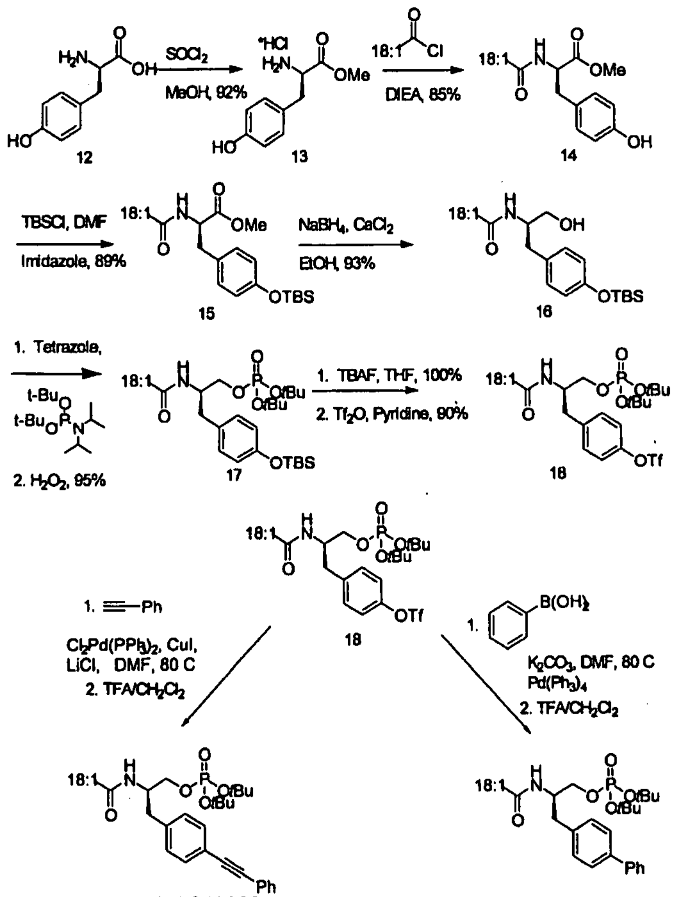
Reagents: (a) i. DIEA, isobutylchloroformate, ii. $NaBH_4$, H_2O; (b) 1H-Tetrazole, N,N-di-t-butyl diisopropylphosphoramidite; (b) i. 1:1 $DIEA/CH_2Cl_2$; ii. Oleoyl chloride; (d) 1:1 TFA/CH_2Cl_2

Scheme 5



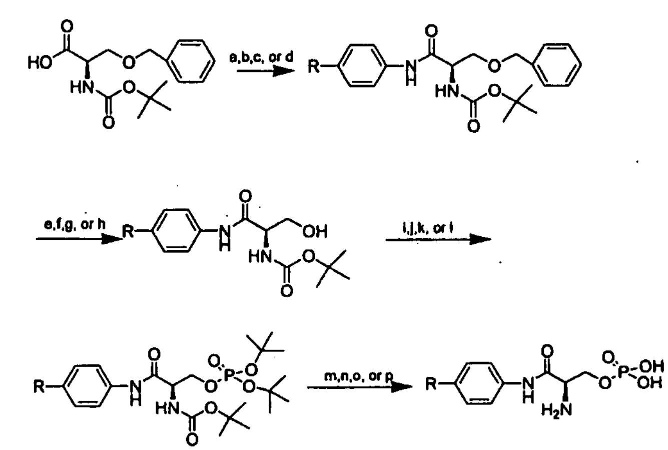
Reagents: (a) $BnNH_2$, $NaBH_3CN$, MeOH; (b) 15% $Pd(OH)_2$, H_2; (c) i. Fmoc-Cl, $NaHCO_3$, THF/H_2O; ii. 1:1 TFA/CH_2Cl_2, then HCl; (d) Oleoyl chloride, DIEA; (e) 1H-Tetrazole, N,N-di-t-butyl diisopropylphosphoramidite; (f) i. piperidine, DMF; ii. 1:1 TFA/CH_2Cl_2

Scheme 6: Synthesis of Tyrosine-based Antagonists



VPC13066 VPC13061

Scheme 7: Synthesis of Amino Analogs



a) PyBOP, DIEA, 4-hexylaniline, 77%
b) PyBOP, DIEA, 4-octylaniline, 73%
c) PyBOP, DIEA, 4-decylaniline, 65%
d) PyBOP, DIEA, 4-dodecylaniline, 71%
e) Di^{t}Bu-di^{i}Pr-phosphoramidite, tetrazole, H_2O_2, 85%
f) Di^{t}Bu-di^{i}Pr-phosphoramidite, tetrazole, H_2O_2, 60%
g) Di^{t}Bu-di^{i}Pr-phosphoramidite, tetrazole, H_2O_2, 70%
h) Di^{t}Bu-di^{i}Pr-phosphoramidite, tetrazole, H_2O_2, 9%

i) H_2, Pd/C, 84%
j) H_2, Pd/C, 96%
k) H_2, Pd/C, 87%
l) H_2, Pd/C, 90%

m) TFA, 77%
n) TFA, 58%
o) TFA, 75%
p) TFA, 77%

VPC22167: R=$(CH_2)_5CH_3$ (a,e,i,m)
VPC22173: R=$(CH_2)_7CH_3$ (b,f,j,n)
VPC22199: R=$(CH_2)_9CH_3$ (c,g,k,o)
VPC22211: R=$(CH_2)_{11}CH_3$ (d,h,l,p)

Example 2

Activity of 2-Substituted LPA analogs at Edg/LPA Receptors

Materials and Methods

Transient Expression in HEK293T Cells

The appropriate receptor plasmid DNA (encoding mouse LPA1, human LPA2 or human LPA3) was mixed with equal amounts of expression plasmids (pcDNA3) encoding a mutated (C351F) rat Gi2a, cow β1, and γ2 proteins and these DNAs were used to transfect monolayers of HEK293T cells (where 'T' indicates expression of the SV-40 virus large T antigen) using the calcium phosphate precipitate method. After about 60 hours, cells were harvested, membranes were prepared, aliquoted and stored at -70°C until use.

GTP[35 γS] Binding:

The GTP[35 γS] assay was performed as described by us previously. Membranes containing 5 ug of protein were incubated in 0.1 ml GTP-binding buffer (in mM: HEPES 50, NaCl 100, $MgCl_2$ 10, pH7.5) containing 5 mg saponin, 10 mM GDP, 0.1 nM GTP[35 γS] (1200 Ci/mmol), and indicated lipid(s) for 30 minutes at 30°C. Samples were analyzed for membrane-bound radionuclide using a Brandel Cell Harvester (Gaithersburg, MD). The C351F mutation renders the Gi2α protein resistant to inactivation by pertussis toxin or the alkylating agent N-ethylmaleimide; however in practice background binding was sufficiently low to obviate these maneuvers.

Measurement of cAMP accumulation:

Assays for cAMP accumulation were conducted on populations of 5 x 10^5 cells stimulated with 10 mM forskolin in the presence of the phosphodiesterase inhibitor isomethylbutylxanthine (IBMX) for 15 minutes at 30° C. cAMP was measured by automated radioimmunoassay.

Measurement of Intracellular Calcium:

A FLIPR ™ (Molecular Devices, Inc.) was used to measure intracellular calcium in A431 and HEK293T cells. A431 cells were seeded (~50,000 cells/well) in 96-well clear bottom black microplates (Corning Costar Corp., Cambridge, MA) and left overnight in CO_2 incubator at 37°C. HEK293T cells were treated likewise, but seeded onto poly D-lysine coated microplates (Becton Dickinson, Franklin Lakes, NJ). A431 cells were dye-loaded with 4 μM Fluo-3 AM ester (Molecular Probes Inc.,

Eugene, OR) in a loading buffer (1x HBSS buffer, pH 7.4, containing 20 mM HEPES, 0.1% BSA, and 2.5 mM probenecid) for 1 hour at 37°C. Cells were then washed four times with the loading buffer and exposed in the FLIPR ™ to sets of compounds. HEK293T cells were loaded with 2 μM Fluo-4 AM ester (Molecular Probes Inc., Eugene, OR) in the same loading buffer without probenecid for 30 minutes and washed four times before being exposed to compounds in the FLIPR ™. In all cases, each concentration of each compound was tested in at least quintuplicate.

Determination of κI:

κI for VPC12249 in experiments were determined by plotting the log of Dose Ratio-1 at each concentration of inhibitor against the log concentration of inhibitor. The X-intercept of the linear transformation is equal to the inverse log of the κI.

Stable expression in RH7777 cells:

Rat hepatoma RH7777 cell monolayers were transfected with the $mLPA_1$ plasmid DNA using the calcium phosphate precipitate method and clonal populations expressing the neomycin phosphotransferase gene were selected by addition of geneticin (G418) to the culture media. The RH7777 cells were grown in monolayers at 37°C in a 5% CO_2/95% air atmosphere in growth media consisting of: 90% MEM, 10% fetal bovine serum, 2 mM glutamine and 1 mM sodium pyruvate.

Cardiovascular Measurements:

All procedures were performed on Male Wistar rats in accordance with National Institutes of Health and University of Virginia animal care and usage guidelines. Anesthesia was induced by 5% halothane (in 100% O_2). Rats were intubated and artificially ventilated with 1.5-1.8% halothane in 100% O_2 for surgical procedures. A femoral artery was cannulated to record mean arterial pressure (MAP) and heart rate (HR), and a femoral vein was cannulated to administer anesthetic agents. A femoral vein was cannulated for administration of lipids. The left splanchnic nerve was isolated via a retroperitoneal approach, and the segment distal to the suprarenal ganglion was placed on two Teflon-coated silver wires that had been bared at the tip (250 mm bare diameter; A-M Systems, Everett, WA). The nerve and wires were embedded in a dental impression material (polyvinylsiloxane; Darby Dental Supply, Westbury, NY), and the wound was closed around the exiting recording wires. On

completion of surgery, the halothane anesthesia was terminated and was replaced by a α-chloralose (30 mg/kg solution in 3% sodium borate; 70 mg/kg initial bolus followed by hourly supplements of 20 mg/kg iv; Fisher Scientific, Pittsburgh, PA). Rats were allowed to stabilize for 45 min before tests began. End-tidal CO_2 was monitored by infrared spectroscopy and was maintained between 3.5 and 4.0%. Body temperature (measured rectally) was maintained at 37°C.

All physiological variables were monitored on a chart recorder (model RS 3600, Gould, Valley View, OH) and simultaneously stored on a videocassette recorder via a digitizer interface (model 3000A, frequency range: DC-22 kHz; Vetter Digital, Rebersburg, VA) for off-line computer analysis. Data was analyzed with Spike 2 (Cambridge Electronics). The MAP was calculated from the pulse pressure measured by a transducer (Statham P10 EZ, Gould) connected to the brachial arterial catheter. The HR was determined by triggering from the pulse pressure (Biotach, Gould). Splanchnic nerve activity (SNA) was filtered (10 Hz-3 kHz band pass with a 60-Hz notch filter), full-wave rectified, and averaged in 1-s bins. The femoral venous catheter (dead space 100mL) was loaded with each lipid and was flushed with 200 mL of saline to expel the drug.

Materials: Chemicals for syntheses were purchased from Aldrich Chemical Company, Inc., Sigma Chemical Company, Inc., Advanced ChemTech Chemical Company, Inc. and/or NovaBiochem Chemical Company, Inc., and were used without further purification. GTP[γ ^{35}S] was purchased from Amerhsam, Fura-3 and Fura-4 AM were purchased from Molecular Probes Inc., A431 and RH7777 cells were purchased from the American Type Culture Collection (Manassas, VA) and tissue culture media and serum was from GibcoBRL/Life Technologies (Gaithersburg, MD). HEK293T cells were a gift from Dr. Judy White's laboratory (Dept. Cell Biology, University of Virginia) while G protein β and γ DNAs were a gift from Dr. Doug Bayliss (Dept. Pharmacology, University of Virginia). LPAs (1-oleoyl and 1-palmitoyl) and dioctyl glyceryl pyrophosphate were purchased from Avanti Polar Lipids (Alabaster, AL).

RESULTS:

Using N-oleoyl ethanolamide phosphoric acid (NAEPA) as a lead structure, a series of 2-substituted LPA analogs was synthesized. The details of the synthesis and analysis of the full set of compounds in this series is described in Example 2. Each compound was characterized by ^{1}H NMR, ^{13}C NMR, and mass spectrometry.

The differential coupling of LPA1 versus LPA2 and LPA3, the lack of a reliable radioligand binding assay, and the near ubiquity of endogenous LPA responses prohibited the use of most common receptor assay techniques (i.e. measurements of adenylyl cyclase activity, calcium mobilization, radioligand binding) to assess each compound's activity. Therefore, we adapted a GTP[γ ^{35}S] binding assay to measure the relative efficacies and potencies of each compound compared to LPA. This assay isolates each recombinant LPA receptor and allows analysis of all three receptors using the same system. Note that membranes from HEK293T cells transfected with only G protein DNAs (i.e., no receptor DNA) were devoid of LPA-stimulated GTP binding despite expressing endogenous LPA receptors.

Many NAEPA compounds with various 2-substituents were synthesized and examined. Since the 2 position is a prochiral site, both enantiomers of the eight compounds were synthesized. Three patterns were revealed when the agonist compounds were tested in this series at the three LPA receptors in the broken cell assay. First, each LPA receptor showed a marked (one log order or more) selectivity for one enantiomer. Second, those compounds with substitutions at the R2 position of Formula I were invariably the more potent agonists. Third, agonist potency decreases as the bulk of the substituent increases. The 2-substituted NAEPA compounds containing either hydrophilic (methylene hydroxy, carbomethyl, methylene amino) or hydrophobic moieties (methyl, ethyl, isopropyl, benzyl) exhibited agonist activity in the GTP[γ ^{35}S] binding assay (see Table 1). The smaller groups conferred greater potency, with the methyl (VPC12086), methylene hydroxy (VPC31143) and methylene amino (VPC12178) compounds being more potent than 1-oleoyl LPA at LPA1 (Table 1). Also, as the 2-substituent becomes bulkier, the efficacy was noticeably reduced at this receptor. In contrast, bulkier hydrophobic side chains, although less potent, were fully efficacious at the LPA2 receptor (Table 1).

Table 1: Agonist activities of 2-substituted N-oleoyl EPA compounds

Lipid	Functional Group	LP A1 EC50 (nM)	LP A1 Emax	LP A2 EC50 (nM)	LP A2 Emax	LP A3 EC50 (nM)	LP A3 Emax
LPA		11.7	100	6.8	100	262.5	100
31143	Methylene Hydroxy	7.9	161.4	116.5	104.6	321.8	106.6
31144	"	>5000	91.1	2645	77.3	4349	N/A
31139	Carbo Methyl	18.5	66.2	29.2	91.3	1484	84.4
31180	"	1215	50.6	3461	60.2	>5000	3.8
12178	Methylene Amino	4.9	98.7	50.3	103.2	683.7	102.9
12048	"	>5000	9.4	>5000	36.4	>5000	3.8
12086	Methyl	3.4	107.6	18.3	95.2	112.6	93.4
12101	"	2900	48.1	>5000	6.9	>5000	9.7
12109	Ethyl	35.8	66.8	161.9	92.6	1083	83.5
12115	"	1580	82.3	4280	85.6	4980	89.3
12098	Isopropyl	73.8	51	799.3	85.8	3815	45.3
40105	"	>5000	14.8	>5000	27.6	>5000	27.7
12084	Benzyl	38.4	23.7	268.3	97	351.4	78.7
12255	"	>5000	N/A	>5000	N/A	>5000	N/A

As was observed with the LPA1 receptor, the small methyl and methylene amino groups conferred the highest potency at the LPA2 receptor but none of these compounds proved more potent than 1-oleoyl LPA at this site. The LPA3 receptor exhibited much the same profile as the LPA2 receptor as far as efficacies and potencies of compounds relative to LPA are concerned. However, the LPA3 receptor characteristically exhibited higher (1-2 log order) EC_{50} values for all compounds, including LPA. Presumably, the LPA3 receptor has an intrinsically lower affinity for LPA and LPA analogs. Like the hydrophilic compounds, each LPA receptor showed strong stereoselectivity for a hydrophobic substituent in the R2 position of Formula I.

Although saturated ligands were repeatedly found that are active at LPA3, mono-unsaturated compounds were also consistently found that are more potent at this receptor. For example, preliminary results with an 18:1 (oleoyl) analog of SDB-213 suggest that the mono-unsaturated compound is significantly more potent (equipotent to LPA) than the saturated compound at LPA3 in this assay. LPA1 and LPA2 do not exhibit this selectivity for mono-unsaturated vs. saturated acyl groups.

To investigate an LPA response in a physiologic context, mean arterial blood pressure (MAP), heart rate, and postganglionic sympathetic tone was monitored in anesthetized adult rats as a function of LPA or LPA analog administration. LPA had been shown previously to increase blood pressure transiently in this model. Intravenous injection of three enantiomeric pairs of compounds resulted in a transient increase in MAP with the same pattern of stereoselectivity as observed with the *in vitro* assays. Concomitant with this rise in MAP was a decrease in heart rate and sympathetic output indicative of baroreceptor reflex response.

The compounds that were only slightly efficacious at LPA1, e.g. the benzyl-containing VPC12084, were assayed for their ability to antagonize LPA induced GTP[γ ^{35}S] binding. Although this compound did block LPA activity in the GTP[γ ^{35}S] binding assay, the benzyl compound (VPC12084) was revealed to posses appreciable agonist activity in assays with greater levels of amplification (e.g., whole cell assays of calcium mobilization or inhibition of cAMP accumulation). In the course of exploring variations of the benzyl substituent, a benzyl-4-oxybenzyl substituent in the same relative configuration (R2: VPC12204) was found to have a reduced, but still measurable, agonist activity in whole cell assays. However, its

enantiomer [i.e., VPC12249, the compound with the benzyl-4-oxybenzyl substituent in the *S* (the R3 substituent of Formula I) configuration] was completely devoid of agonist activity in the whole cell assays and in the GTP[γ ^{35}S] binding assay.

VPC12249 was tested for its ability to block LPA-induced GTP[γ ^{35}S] binding at each recombinant LPA receptor. As is shown by the rightward, parallel shifts in the concentration response curves as a function of VPC12249 concentration, this compound is a surmountable antagonist at the LPA1 and LPA3, but not the LPA2, receptors (Fig. 6). The K_I values for VPC12249 determined by Schild regression are 137 and 428 nM at the LPA1 and LPA3 receptors, respectively, in this assay. The same activity was determined with human LPA1 using a recombinant baculovirus-infected insect Sf9 cell membrane preparation.

The antagonist activity measured in the broken cell assays was confirmed in whole cell experiments wherein LPA-induced rises in free intracellular calcium in HEK293T cells were blocked. This cell type expresses the LPA1 and LPA3, but not LPA2 receptor genes, as determined by RT-PCR. Increasing concentrations of VPC12249 resulted in parallel, rightward shifts in the LPA concentration response curves (K_I 132 nM). The extent of rightward shift observed in the same experimental protocol with A431 cells, which express the LPA2 as well as the LPA1 and LPA3 genes (RT-PCR not shown), was much smaller (K_I 1970 nM) as predicted from the lack of antagonist activity of VPC12249 at the calcium-mobilizing LPA2 receptor in the GTP binding assay. The blocking action of VPC12249 was not a general post-receptor event as shown by the lack of antagonism of ATP-evoked calcium transients. Inhibition of forskolin-induced increases in cAMP levels in RH7777 cells stably expressing LPA1 was also inhibited by VPC12249.

Example 3

Synthesis of *rac*-N-oleoyl-1-hydroxy-propylamide phosphonic acid (NOHPP)

Two batches of NOHPP have been synthesized as a racemic mixture. After observing LPA mimetic activity with the first batch (named JAR1842), the material was re-synthesized, and this batch was named VPC12031. Both batches showed the same profile on thin layer chromatography (TLC) (R_f 0.31, chloroform/acetone/methanol/glacial acetic acid/water, 50/15/13/12/4) whereon they migrated as a single, discrete spot. Further, the NMR spectrum of VPC12031 was as expected and the formula weight measured by mass spectrometry (419.5 daltons) agrees with that of the structure. Thus confirming the identity and high degree of purity of the NOHPP sample.

To assay NOHPP and other compounds at individual LPA receptors, the adapted GTPγS binding assay of Example 3 was used. Briefly, individual, recombinant LPA receptors were express along with heterotrimeric G proteins in HEK293T cells. For the LPA receptor LPA1, which naturally couples to Gi/o proteins, membranes from rat hepatoma cells (RH7777) were also used that have been transfected only with LPA1 DNA. Membranes prepared from these cells are used to measure GTPγ[^{35}S] binding as a function of test compound concentration. The resulting dose-response curves provide relative potency (EC_{50}) and efficacy (E_{max}) for the test compounds. The assay is robust and free of background from the LPA receptors endogenous to HEK293T cells. In Figures 2A-C we show the relative activities of LPA and NOHPP at the three known (i.e. cloned) LPA receptors (LPA1, LPA2 and LPA3). NOHPP is distinctly less potent and far less efficacious than LPA at LPA3, but as efficacious as, but somewhat less potent than, LPA at LPA1 and LPA2.

NOHPP was also tested for activity at the three cloned LPPs. Although the phosphonate head group eliminates the possibility of NOHPP acting as a substrate, it is possible that the compound could be a competitive inhibitor. For the initial assay, 0.1, 1.0 and 10.0 mM NOHPP was tested in competition with 50 nM [^{32}P]LPA and assayed for the appearance of water soluble radionuclide. As can be seen in Figure 3, NOHPP inhibited LPP3 and, to a lesser extent, LPP1. NOHPP was inactive in this assay at LPP2.

The activity of the alpha keto NOHPP analog (VPC12060) at the three LPPs has also been tested. The structure of the alpha keto NOHPP analog is as follows:

Figure 4 presents data concerning the inhibition of the three phosphatases (PAP2a, PAP2b and PAP2c (aka LPP1, LPP3, LPP2, respectively) by the alpha hydroxy phosphonate analog of LPA(VPC12031) and the alpha keto phosphonate analog of LPA (wls060). Both compounds are markedly better at the PAP2b (LPP3) than PAP2a (LPP1) which in turn is better than PAP2c (LPP2). Based on the results of the activity of NOHPP, it is anticipated that analogs of NOHPP will also be active.

Example 4

Synthesis of NOHPP [(9Z)-N-(3-hydroxypropyl)octadec-9-enamide]

To a solution of 1-aminopropanol (4.04 ml, 52.8 mmol) and pyridine (4.27 ml, 52.8 mmol) in methylene chloride was slowly added oleoyl chloride (5 ml, 15.1 mmol). After 2 hours, the mixture was diluted with chloroform and washed with ammonium chloride (3x), brine (3x) and dried over sodium sulfate. Column chromatography (15% acetone/chloroform to 50% acetone/chloroform) provided the product as white solid.

(9Z)-N-(3-oxopropyl)octadec-9-enamide **(22)**

To a suspension of pyridinium chlorochromate (0.477g, 2.21 mmol) and sodium acetate (36 mg, 0.44 mmol) in 10 ml of dichloromethane was cannulated as solution of 1 in 8 ml of dichloromethane. The mixture was stirred for 10 hours, quenched with ether, then filtered through celite and concentrated to an oil. Chromatography in 15% acetone/chloroform provided the product in 47% yield (0.495 g).

(9Z)-N-{3-[bis(tert-butoxy)carbonyl]-3-hydroxypropyl}octadec-9-enamide **(23)**

To a suspension of sodium hydride in 8 ml of THF at 0°C was cannula as a solution aldehyde 3 in 6 ml THF. The mixture was allowed equilibrate to room temperature over an hour. The reaction was quenched with water and acidified with 10% HCl followed by extraction with chloroform (3x). The combined organic extracts were washed with brine (3x) and dried over sodium sulfate. Chromatography in 15% acetone/chloroform provided the product as off-white solid (130 mg, 36%).

(9Z)-N-(rac-3-hydroxy-3-phosphonopropyl)octadec-9-enamide **(24)**

To a solution of **23** in 1 ml of dichloromethane was added 0.3 ml trifluoroacetic acid. The mixture was stirred for 4 hours (monitored by TLC). Concentration and repeated washings with ether provided the product in 100% yield.

Example 5

Synthesis of wls-b8L

3-(diethoxycarbonyl)propanenitrile:

In a 50 ml of dry ethanol was added sodium (1.38 g, 60 mmol). After complete dissolution of sodium, diethyl phosphite (7.75 ml, 60 mmol) dissolved in 20 ml toluene was added slowly. After stirring for 1 hour at room temperature, acrylonitrile (3.95 ml, 57 mmol) dissolved in 20 ml toluene was added dropwise over 1 hour. The mixture was allowed to stir overnight. Dilution with water, extraction with CH_2Cl_2 (3X) and drying with sodium sulfate provided a crude yellow oil, which was concentrated and distilled under vacuum (134 °C, 1.5 mmHg) to provide the product as clear liquid (6.11 g, 53 %).

(3-aminopropyl)diethoxyphosphino-1-one:

To a suspension of 3-(diethoxycarbonyl)propanenitrile (2.78 g, 14.5 mmol) and cobalt(II) chloride (0.378 g, 2.91 mmol) in methanol at -30 °C was added sodium borohydride (5.49 g, 145 mmol) in small portions. The mixture was allowed to equilibrate to room temperature and stirred overnight. Concentrated HCl was added until the black mixture turned blue, which was extracted twice with dichloromethane. The pH of the aqueous phase was adjusted to 9 by addition of concentrated ammonium hydroxide, and the solution was evaporated to dryness to generate pink/blue solid. Concentrated ammonium hydroxide (100 ml) and dichloromethane (200 ml) was added and stirring was continued for 1 hour.

Extraction with CH_2Cl_2 (2X), filtration, drying over sodium sulfate, and concentration provided the product as yellow liquid (2.0 g, 70%).

(9Z)-N-[3-(diethoxycarbonyl)propyl]octadec-9-enamide:

To a solution of (3-aminopropyl)diethoxyphosphino-1-one (0.736 g, 3.77 mmol) and pyridine (0.61 ml, 7.54 mmol) at 0 °C was slowly added oleoylchloride (1.25 ml, 3.77 mmol) over 20 minutes. The reaction mixture was allowed to warm to room temperature, followed by stirring for another 2 hours. Dilution with ethyl acetate and extraction with saturated aqueous ammonium chloride (3X) and brine (3X), drying over sodium sulfate, and flash chromatography using 15% acetone/chloroform provided the product as off-white solid (0.572 g, 33%).

(9Z)-N-(3-phosphonopropyl)octadec-9-enamide:

To a solution of (9Z)-N-[3-(diethoxycarbonyl)propyl]octadec-9-enamide (0.219 g, 0.48 mmol) in acetonitrile was added trimethylsilylbromide (0.189 ml, 1.43 mmol). The reaction mixture was refluxed for 2 hours and the solvent was evaporated under reduced pressure. Addition of ether and removal in *vacuo*, a process repeated several times, provided the product as brown solid (0.192 g, 100%).

Example 6

Analysis of wls-b8L activity:

To assay wls-b8L at individual LPA receptors, the GTPγS binding assay described in Example 2 was utilized. Briefly, individual recombinant LPA receptors were expressed along with heterotrimeric G proteins in HEK293T cells. Membranes prepared from these cells are used to measure GTPγ(^{35}S] binding as a function of test compound concentration. The resulting dose-response curves provide relative potency (EC_{50}) and efficacy (E_{max}) for the test compounds. wls-b8L was found to be more potent and efficacious at LPA1 than at either LPA2 or LPA3. Also evident is the lack of receptor-type selectivity by the other phosphonate compounds.

wls-b8L and the other phosphonates were also tested also for activity in three whole cell assays. For the LPA receptor LPA1, which naturally couples to Gi/o proteins, rat hepatoma cells (RH7777) were used that have been transfected only with mouse LPA1 DNA. In these cell cultures, drug dependent inhibition of cAMP accumulation was measured. wls-b8L, was determined to be fully efficacious in this

assay at a concentration of 1 μM. At this concentration wls-b8L is inactive at mobilizing calcium through endogenous receptors LPA receptors expressed by MDA MB-231 cells. Finally, the activity of wls-b8L in activating a chloride conductance in *Xenopus laevis* ooctyes was measured. These cells are normally activated by LPA through an endogenous receptor. However, wls-b8L does not evoke this response. However, when injected with human LPA3 or LPA2 mRNAs a very modest response to wls-b8L is detected, but only when a concentration of 10 μM is applied to the oocyte surface.

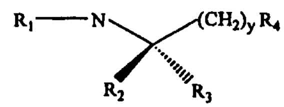
Example 7

Analysis of VPC12249 Effects in a Renal Ischemia-reperfusion Model

To examine the effect of the compound VPC12249 on renal injury following ischemia-reperfusion (IR) the following experiment was conducted. C57BL6 mice (20 gm, 8 wks of age) were used for all studies. The surgical protocol of renal IR has been previously described (Okusa et al., 1999. Am J. Physiol. (Renal Physiol.) 277: F404-412; Okusa, et al., 2000 Am.J. Physiol. (Renal Physiol.) 279; F809-F818, and Okusa et al., 2001 Kidney Int. 59:2114-2125). For the injury protocol, bilateral flank incisions were made under anesthesia with a regimen that consists of ketamine (100 mg/kg, ip.), xylazine (10 mg/kg, ip.) and acepromazine (1 mg/kg, im.). Renal pedicles were exposed and cross-clamped for 27-32 min. Kidneys were examined for immediate reperfusion following clamp removal. Wounds were closed and mice were returned to cages. Mice were injected with VPC12249 (0.01, 0.1 and 1.0 mg/kg/2hrs) or vehicle. The mice were sacrificed and the kidneys harvested at 1, 4, 8, 24, 48 hrs, 5 and 7 days. Treatment with VPC12249 or vehicle was initiated 2 hrs prior to ischemia, at the time of reperfusion and every 2 hrs for 4 additional doses. Mice were sacrificed following 24 hrs of reperfusion and plasma was obtained for BUN and creatinine. Plasma creatinine was 1.49±0.26 (n=4), 1.06±0.25 (n=4), 0.45±±0.13 (n=4) and 0.31±0.4 (n=4), for vehicle, VPC12249 0.01, 0.1 and 1.0 mg/kg/2hrs, respectively. VPC12249 administered at 0.1 ($P<0.05$) and 1.0 mg/kg/2hrs ($P<0.1$) were significantly different compared to vehicle. H and E sections revealed a marked degree of tissue preservation following VPC12249 at 1 mg/kg/2hr.

Claims

1. A compound represented by the formula:



wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl, C_8-C_{22} alkenyl, C_8-C_{22} alkanoyl, C_8-C_{22} alkenoyl,

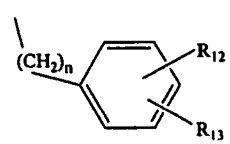
$$-\overset{\displaystyle O}{\overset{\|}{C}}(CH_2)_m-Z-R_{11} \quad \text{and} \quad -(CH_2)_m-Z-R_{11} \;;$$

wherein m is 0-20;

Z is selected from the group consisting of C_3-C_{10} cycloalkyl, C_3-C_{15} bicycloalkyl, C_3-C_{10} heterocyclic and aryl;

R_{11} is selected from the group consisting of C_1-C_{10} alkyl, C_1-C_{20} alkoxyl, C_1-C_{20} alkylthio, and C_1-C_{20} alkylamino;

R_2 and R_3 are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, -(C_1-C_4 alkyl)OH, -(C_1-C_4 alkyl)NH_2, -$COOR_5$, -(C_1-C_4 alkyl)$COOR_5$, -(C_1-C_{10} alkyl)aryl, C_3-C_8 cycloalkyl, C_3-C_8 heterocyclic, C_7-C_{12} bicyclic, (C_5-C_8 alkyl)aryl, (C_5-C_8 alkenyl)aryl, (C_5-C_8 alkynyl)aryl, and



wherein n is 0-10;

R_5 is selected from the group consisting of H and C_1-C_4 alkyl;

R_{12} is selected from the group consisting of halo, C_1-C_{10} alkyl, (C_0-C_{12} alkyl)aryl, (C_2-C_{12} alkenyl)aryl, (C_2-C_{12} alkynyl)aryl, -(C_1-C_4 alkyl)OH, -(C_2-C_{12} alkenyl)OH, SR_6, SOR_6, NHR_6 and OR_6;

R_{13} is selected from the group consisting of H, halo, C_1-C_{10} alkyl, (C_0-C_{12} alkyl)aryl, (C_2-C_{12} alkenyl)aryl, (C_2-C_{12} alkynyl)aryl, -(C_1-C_4 alkyl)OH, -(C_2-C_{12} alkenyl)OH, SR_6, SOR_6, NHR_6 and OR_6;

wherein R_6 is selected from the group consisting of C_1-C_{16} alkyl, C_2-C_{16} alkenyl, C_2-C_{16} alkynyl, -(C_1-C_4 alkyl)R_7, -(C_2-C_4 alkenyl)R_7, -(C_1-C_4 carboxy)R_7 and -(C_2-C_4 alkynyl)R_7; and

R_7 is selected from the group consisting of optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_3-C_8 heterocyclic, optionally substituted C_7-C_{12} bicyclic and optionally substituted C_5-C_8 cycloalkenyl and optionally substituted aryl;

y is 1-4; and

R_4 is selected from the group consisting of hydroxyl, phosphate, methylene phosphonate, α-substituted methylene phosphonate and thiophosphate.

2. The compound of claim 1 wherein

R_1 is selected from the group consisting of

$$-\overset{\displaystyle O}{\overset{\|}{C}}(CH_2)_m-Z-R_{11} \quad \text{and} \quad -(CH_2)_m-Z-R_{11} \;;$$

R_{13} is H;

R_4 is selected from the group consisting of hydroxyl, phosphate and methylene phosphonate, α-substituted methylene phosphonate and thiophosphate; and

y is 1.

3. The compound of claim 1 wherein

R_1 is selected from the group consisting of C_8-C_{22} alkyl, C_8-C_{22} alkenyl, C_8-C_{22} alkanoyl, and C_8-C_{22} alkenoyl;

R_{13} is H; and

R_4 is selected from the group consisting of hydroxyl, phosphate and methylene phosphonate α-substituted methylene phosphonate and thiophosphate; and

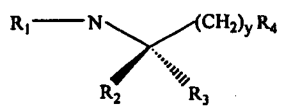
y is 1.

4. The compound of claim 3 wherein

n is 1; and

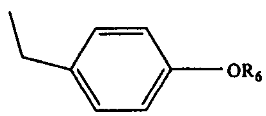
R_{12} is selected from the group consisting of SR_6, SOR_6, NHR_6 and OR_6.

5. A compound represented by the formula:



wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl, C_8-C_{22} alkenyl;

R_2 is selected from the group consisting of C_1-C_3 alkyl, -(C_2-C_4 alkyl)OH, -(C_1-C_4 alkyl)NH_2, -(C_1-C_4 alkyl)$COOR_5$, -(C_1-C_4 alkyl)aryl, and



R_4 is selected from the group consisting of hydroxyl, phosphate, methylene phosphonate, α-substituted methylene phosphonate and thiophosphate;

R_5 is selected from the group consisting of H and C_1-C_4 alkyl; and

R_6 is selected from the group consisting of C_3-C_{16} alkyl, C_3-C_{16} alkenyl, and -(C_1-C_4 alkyl)R_7;

R_7 is selected from the group consisting of C_3-C_8 cycloalkyl, C_3-C_8 heterocyclic, C_7-C_{12} bicyclic, C_5-C_8 cycloalkenyl and aryl; and

y is 1-4.

6. The compound of claim 5 wherein

R_1 is C_{13}-C_{17} alkyl or C_{17}-C_{21} alkenyl; and

R_4 is phosphate, methylene phosphonate or α-substituted methylene phosphonate; and

y is 1.

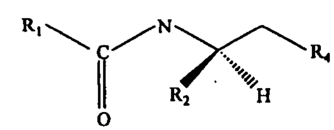
7. The compound of claim 6 wherein

R_2 is selected from the group consisting of C_1-C_3 alkyl, methylene amino, methylene alkyne, phenyl, benzyl, methylene furan, methylene-2-naphthalene, methylene phenol, methylene amino benzyl, methylene phenyl-4-O-benzyl, methylene phenyl-4-benzyl, methylene phenyl-4-chloro, methylene phenyl-4-*trans*-styrene, methylene phenyl-4-*cis*-styrene, methylene phenyl-4-O-2,6-dichlorobenzyl and methylene phenyl-4-phenyl.

8. The compound of claim 6 wherein R_4 is phosphate and R_2 is selected from the group consisting of C_1-C_3 alkyl and benzyl.

9. A composition comprising the compound of claim 1 and a pharmaceutically acceptable carrier.

10. An LPA receptor agonist represented by the formula:



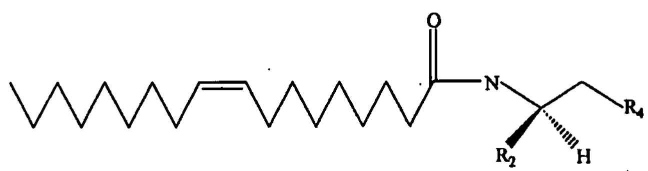
wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl, C_8-C_{22} alkenyl;

R_2 is selected from the group consisting of C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, -(C_2-C_4 alkyl)OH, -(C_1-C_4 alkyl)NH_2, -(C_1-C_4 alkyl)$COOR_5$ and aryl;

R_4 is selected from the group consisting of hydroxyl, phosphate and methylene phosphonate and α-substituted methylene phosphonate; and

R_5 is selected from the group consisting of H and C_1-C_4 alkyl.

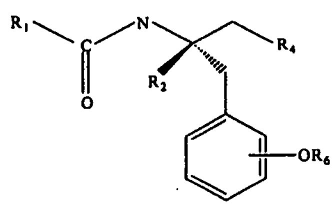
11. The LPA receptor agonist of claim 10 wherein the agonist has the general structure:



wherein R_2 is methyl, ethyl, propyl, isopropyl, butyl, methylene amino, methylene alkyne, phenyl, benzyl, methylene furan, methylene-2-naphthalene; and R_4 is hydroxyl (OH), phosphate (PO_4), or methylene phosphonate (CH_2PO_3).

12. A composition comprising the compound of claim 10 and a pharmaceutically acceptable carrier.

13. An LPA receptor antagonist represented by the formula:



wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl and C_8-C_{22} alkenyl;

R_2 is selected from the group consisting of H, C_1-C_4 alkyl;

R_4 is selected from the group consisting of hydroxyl, phosphate and methylene phosphonate;

R_6 is selected from the group consisting of C_1-C_{16} alkyl, C_1-C_{16} alkenyl, -(C_1-C_4 alkyl)R_7; and

R_7 is selected from the group consisting of C_3-C_8 cycloalkyl, C_3-C_8 heterocyclic, C_7-C_{12} bicyclic, C_3-C_8 cycloalkenyl and aryl.

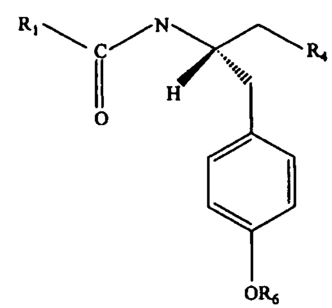
14. The compound of claim 13 wherein

R_1 is C_{13}-C_{17} alkyl or C_{17}-C_{21} alkenyl;

R_2 and R_3 are H; and

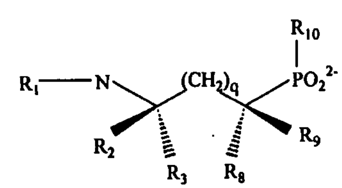
R_4 is phosphate, methylene phosphonate or α-substituted methylene phosphonate.

15. The compound of claim 14 wherein the agonist is represented by the formula:



16. The compound of claim 13 wherein R_2 and R_3 are independently selected from the group consisting of H, benzyl, methylene furan, methylene-2-naphthalene, methylene phenyl-4-O-benzyl, methylene phenyl-4-benzyl, methylene phenyl-4-chloro, methylene phenyl-4-*trans*-styrene, methylene phenyl-4-*cis*-styrene, methylene phenyl-4-O-2,6-dichlorobenzyl and methylene phenyl-4-phenyl.

17. A lyso-lipid phosphate phosphatase resistant LPA analog, said agonist represented by the formula



wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl, C_8-C_{22} alkenyl, C_8-C_{22} alkanoyl, C_8-C_{22} alkenoyl,

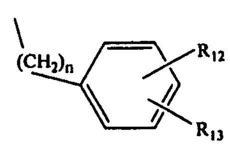
$$-\overset{\overset{\displaystyle O}{\|}}{C}(CH_2)_m-Z-R_{11} \quad \text{and} \quad -(CH_2)_m-Z-R_{11} \;;$$

wherein m is 0-20;

Z is selected from the group consisting of C_3-C_{10} cycloalkyl, C_3-C_{15} bicycloalkyl, C_5-C_{10} heterocyclic and aryl;

R_{11} is selected from the group consisting of C_1-C_{10} alkyl, C_1-C_{20} alkoxyl, C_1-C_{20} alkylthio, and C_1-C_{20} alkylamino;

R_2 and R_3 are independently selected from the group consisting of H, hydroxy, C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, -(C_1-C_4 alkyl)OH, -(C_1-C_4 alkyl)NH_2, -$COOR_5$, -(C_1-C_4 alkyl)$COOR_5$, -(C_1-C_4 alkyl)aryl, C_3-C_8 cycloalkyl, C_3-C_8 heterocyclic, C_7-C_{12} bicyclic, (C_5-C_{10} alkyl)aryl, (C_5-C_8 alkenyl)aryl, (C_5-C_8 alkynyl)aryl, and



wherein n is 0-10;

R_5 is selected from the group consisting of H and C_1-C_4 alkyl;

R_{12} is selected from the group consisting of halo, C_1-C_{10} alkyl, (C_0-C_{12} alkyl)aryl, (C_2-C_{12} alkenyl)aryl, (C_2-C_{12} alkynyl)aryl, -(C_1-C_4 alkyl)OH, -(C_2-C_{12} alkenyl)OH, SR_6, SOR_6, NHR_6 and OR_6;

R_{13} is selected from the group consisting of H, halo, C_1-C_{10} alkyl, (C_0-C_{12} alkyl)aryl, (C_2-C_{12} alkenyl)aryl, (C_2-C_{12} alkynyl)aryl, -(C_1-C_4 alkyl)OH, -(C_2-C_{12} alkenyl)OH, SR_6, SOR_6, NHR_6 and OR_6;

wherein R_6 is selected from the group consisting of C_1-C_{16} alkyl, C_2-C_{16} alkenyl, C_2-C_{16} alkynyl, -(C_1-C_4 alkyl)R_7, -(C_2-C_4 alkenyl)R_7, -(C_1-C_4 carboxy)R_7 and -(C_2-C_4 alkynyl)R_7; and

R_7 is selected from the group consisting of optionally substituted C_3-C_8 cycloalkyl, optionally substituted C_3-C_8 heterocyclic, optionally substituted C_7-C_{12} bicyclic, optionally substituted C_3-C_8 cycloalkenyl and optionally substituted aryl;

q is 1-4;

R_8 and R_9 are independently selected from H, hydroxyl, amino, COOH, halo, -PO_3; or R_8 and R_9 taken together form a keto group or a methylene group; and

R_{10} is selected from the group consisting of O, S and NH.

18. The compound of claim 17 wherein

R_1 is selected from the group consisting of

$$-\overset{\overset{\displaystyle O}{\|}}{C}(CH_2)_m-Z-R_{11} \quad \text{and} \quad -(CH_2)_m-Z-R_{11} \;;$$

R_{13} and R_{10} are H; and

q is 1.

19. The compound of claim 17 wherein

R_1 is selected from the group consisting of C_8-C_{22} alkyl, C_8-C_{22} alkenyl, C_8-C_{22} alkanoyl, and C_8-C_{22} alkenoyl;

R_{13} and R_{10} are H; and

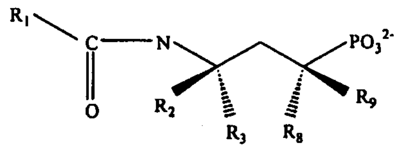
q is 1.

20. The compound of claim 19 wherein

n is 1; and

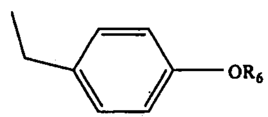
R_{12} is selected from the group consisting of SR_6, SOR_6, NHR_6 and OR_6

21. A lyso-lipid phosphate phosphatase resistant LPA analog, said agonist represented by the formula



wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl and C_8-C_{22} alkenyl;

R_2 and R_3 are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, -(C_1-C_4 alkyl)OH, -(C_1-C_4 alkyl)NH_2, -$COOR_5$, -(C_1-C_4 alkyl)$COOR_5$, and



R_5 is selected from the group consisting of H and C_1-C_4 alkyl; and

R_6 is selected from the group consisting of C_3-C_{16} alkyl, C_3-C_{16} alkenyl, -(C_1-C_4 alkyl)R_7;

R_7 is selected from the group consisting of C_3-C_8 cycloalkyl, C_3-C_8 heterocyclic, C_7-C_{12} bicyclic and C_5-C_8 aryl;

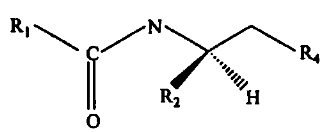
R_8 and R_9 are independently selected from the group consisting of H, C_1-C_4 alkyl, and -PO_3, or R_8 and R_9 are combined to form a keto group.

22. The analog of claim 21 wherein

R_1 is C_{13}-C_{17} alkyl or C_{17}-C_{21} alkenyl; and

R_3, R_8 and R_9 are H.

23. A lyso-lipid phosphate phosphatase resistant LPA receptor agonist represented by the formula:



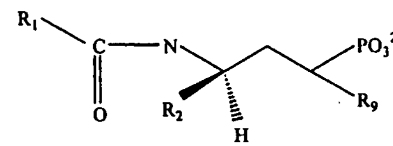
wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl and C_8-C_{22} alkenyl;

R_2 is selected from the group consisting of H, hydroxyl, C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, -(C_1-C_4 alkyl)OH, -(C_1-C_4 alkyl)NH_2, -$COOR_5$, -(C_1-C_4 alkyl)$COOR_5$, and benzyl; and

R_9 is selected from the group consisting of H, C_1-C_4 alkyl, halo and -PO_3.

24. A composition comprising the compound of claim 17 and a phamaceutically acceptable carrier.

25. A method of enhancing wound repair, said method comprising the steps of contacting the wound with a composition comprising a compound represented by the formula:



wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl and C_8-C_{22} alkenyl;

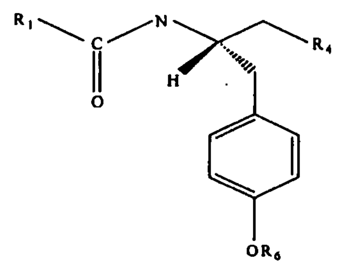
R_2 is selected from the group consisting of C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, -(C_2-C_4 alkyl)OH, -(C_1-C_4 alkyl)NH_2, -(C_1-C_4 alkyl)$COOR_5$ and benzyl;

R_4 is selected from the group consisting of hydroxyl, phosphate and methylene phosphonate; and

R_5 is selected from the group consisting of H and C_1-C_4 alkyl.

26. The method of claim 25 wherein R_2 is methyl, ethyl, propyl, isopropyl, butyl, methylene amino, methylene alkyne, phenyl, benzyl, methylene furan, methylene-2-naphthalene; and R_4 is hydroxyl (OH), phosphate (PO_4), methylene phosphonate (CH_2PO_3) or α-substituted methylene phosphonate.

27. A method of treating a disease characterized by cell hyper proliferation, said method comprising the step of administering to a patient a composition comprising a compound represented by the formula



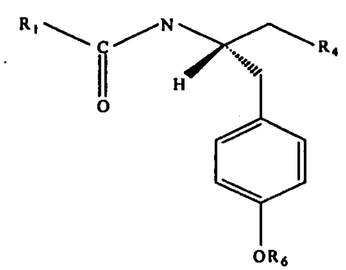
wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl and C_8-C_{22} alkenyl;

R_4 is selected from the group consisting of hydroxyl, phosphate and methylene phosphonate;

R_6 is selected from the group consisting of C_3-C_{16} alkyl, C_3-C_{16} alkenyl, -(C_1-C_4 alkyl)R_7; and

R_7 is selected from the group consisting of C_3-C_8 cycloalkyl, C_3-C_8 heterocyclic, C_7-C_{12} bicyclic, C_5-C_8 cycloalkenyl and aryl, with the proviso that R_2 and R_3 are not both H.

28. A method of treating reperfusion type injury, said method comprising the step of administering to a patient a composition comprising a compound represented by the formula



wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl and C_8-C_{22} alkenyl;

R_4 is selected from the group consisting of hydroxyl, phosphate and methylene phosphonate;

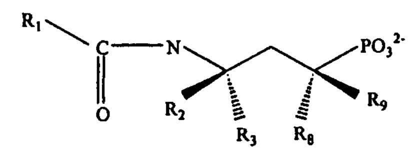
R_6 is selected from the group consisting of C_3-C_{16} alkyl, C_3-C_{16} alkenyl, -(C_1-C_4 alkyl)R_7; and

R_7 is selected from the group consisting of C_3-C_8 cycloalkyl, C_3-C_8 heterocyclic, C_7-C_{12} bicyclic, C_5-C_8 cycloalkenyl and aryl, with the proviso that R_2 and R_3 are not both H.

29. The method of claim 28 wherein the composition is administered prior to injury to prevent tissue damage.

30. The method of claim 28 wherein the composition is administered after injury to prevent or limit tissue damage.

31. A method of inhibiting LPP activity, said method comprising the step of administering a compound represented by the formula



wherein R_1 is selected from the group consisting of C_8-C_{22} alkyl and C_8-C_{22} alkenyl;

R_2 and R_3 are independently selected from the group consisting of H, C_1-C_6 alkyl, C_2-C_4 alkenyl, C_2-C_4 alkynyl, -(C_1-C_4 alkyl)OH, -(C_1-C_4 alkyl)NH_2, -$COOR_5$, and -(C_1-C_4 alkyl)$COOR_5$;

R_5 is selected from the group consisting of H and C_1-C_4 alkyl; and

R_8 and R_9 are independently selected from the group consisting of H, halo, C_1-C_4 alkyl, and -PO_3, or R_8 and R_9 are combined to form a keto group.

32. The method of claim 31 wherein R_2 is H

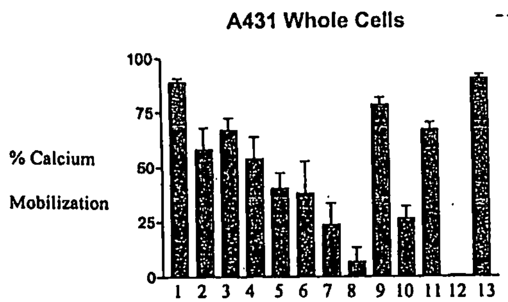


Fig. 1

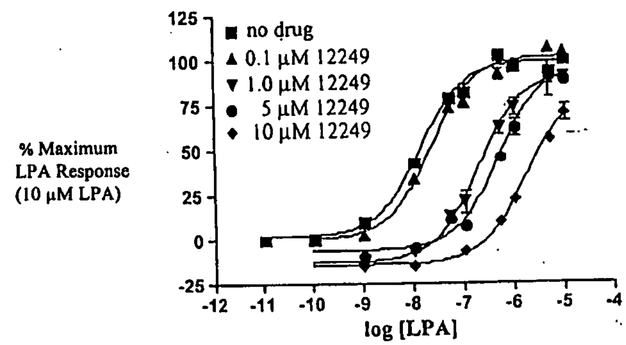


Fig. 2A

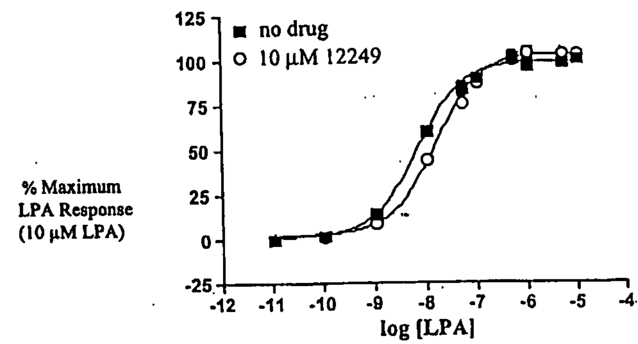


Fig. 2B

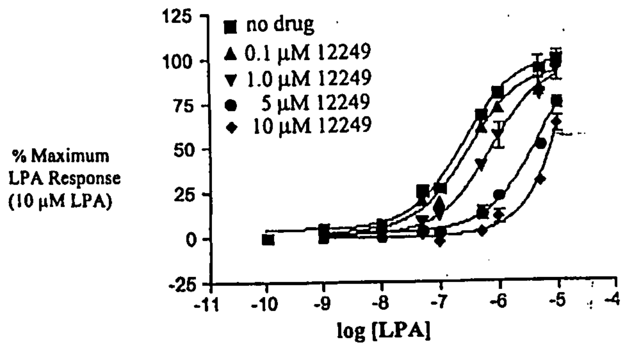


Fig. 2C

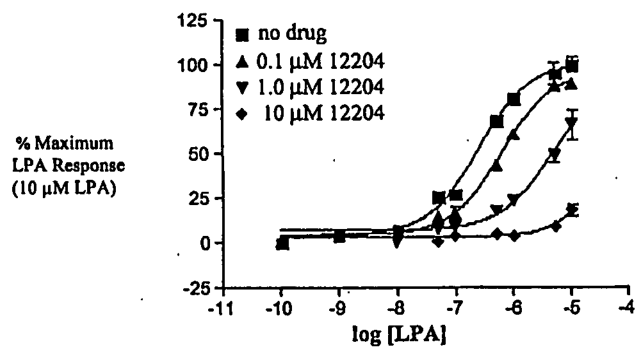


Fig. 2D

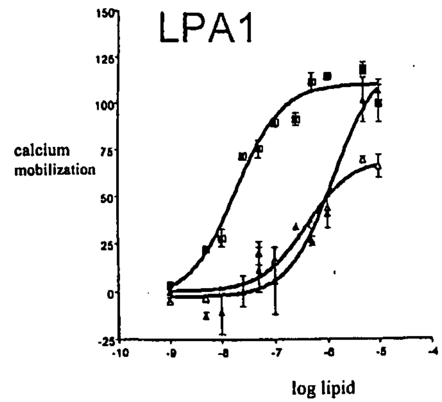


Fig. 3A

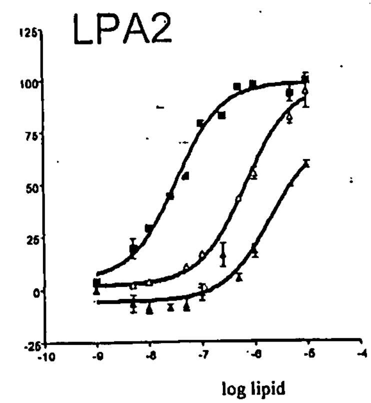


Fig. 3B

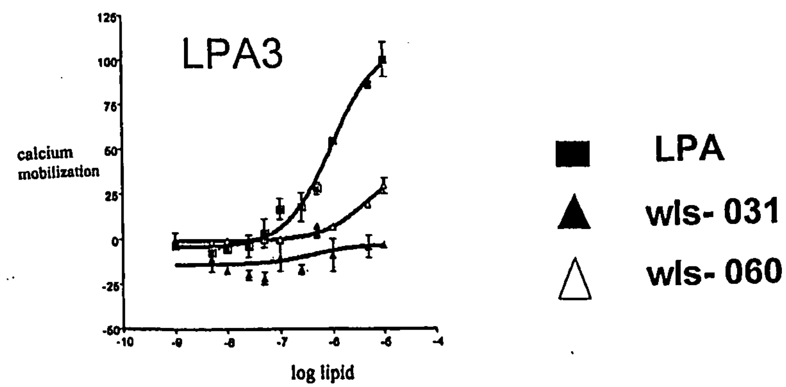


Fig. 3C

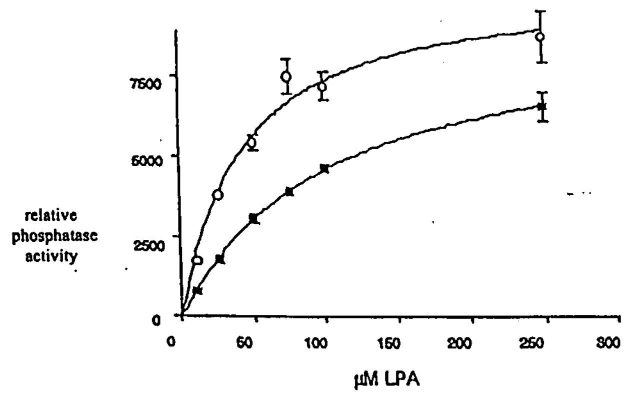


Fig. 4A

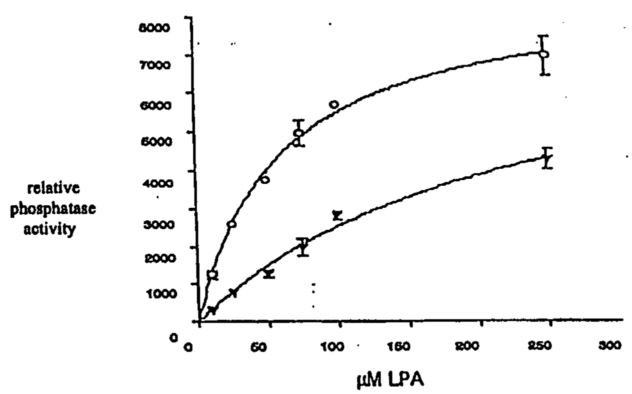


Fig. 4B

O No Inhibitor ■ + 500 μM VPC 12031 ▼ + 500 μM VPC 12060

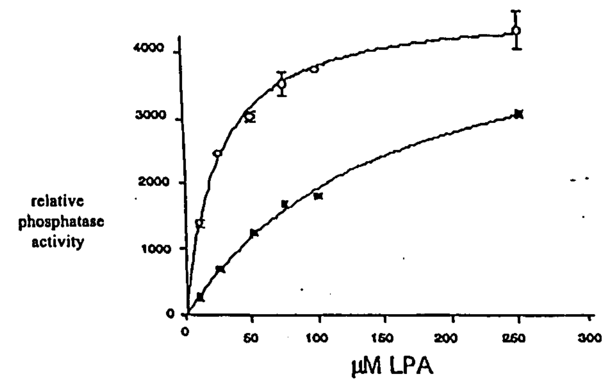


Fig. 5A

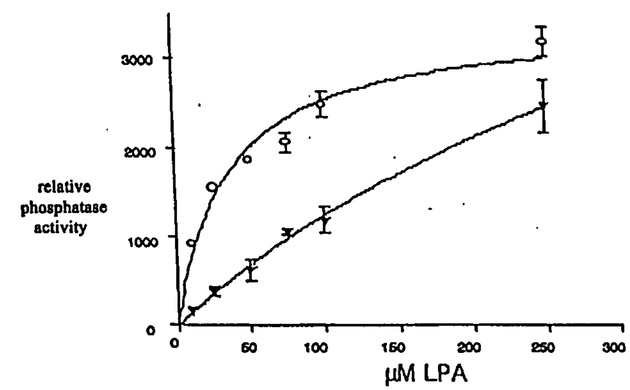


Fig. 5B

O No Inhibitor ■ + 500 μM VPC 12031 ▼ + 500 μM VPC 12060

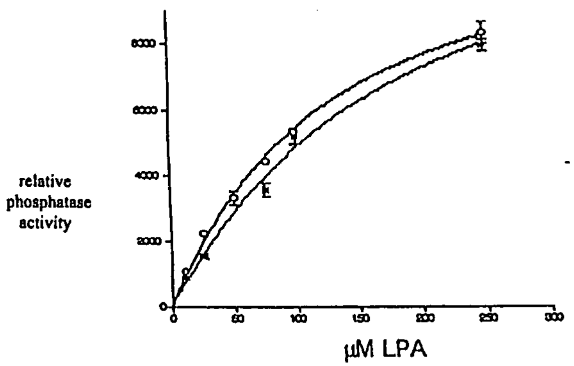


Fig. 6A

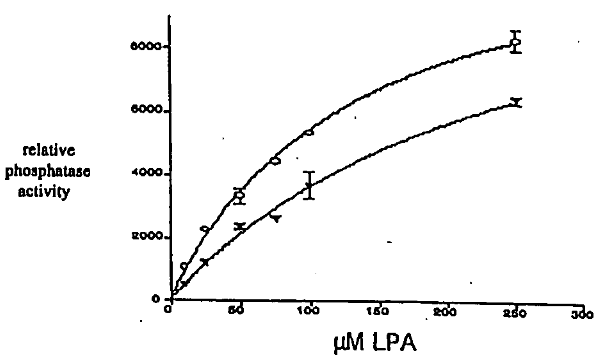


Fig. 6B

○ No Inhibitor ■ + 500 µM VPC 12031 ▼ + 500 µM VPC 12060